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INFLUENCE OF SOIL ENVIRONMENT ON THE ROOT-ROT OF TOBACCO

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INTRODUCTION

The foremost considerations in connection with the study of disease in plants are the pathogenicity of the parasite, the susceptibility of the host, and the environmental conditions favoring the infection and progress of the parasite. It is well known, however, that the relative pathogenicity of the parasite and susceptibility of the host are not always easily distinguishable one from the other in disease, and that they are largely influenced by environmental conditions. To the practical grower environmental conditions have been considered as all important, to the exclusion of the parasite, while, on the other hand, the tendency in the past on the part of pathologists and botanists has been to devote a great deal of energy to the study of the parasite, with only passing interest being given to the influence of the environment on disease, as recently emphasized by Jones (*16*).[2] This is especially true of plant diseases having their origin or region of attack on underground portions of plants. The literature upon actual experimental data with reference to the influence of soil conditions upon a soil-infesting parasite is fragmentary, and for the most part concerned with only one or two variable factors, so that the conclusions can not always be relied upon because of failure to give due consideration to other factors perhaps even more influential in the end result obtained. The Thielavia-rootrot of tobacco (*Nicotiana tabacum*) forms a relationship of host and parasite apparently admirably adapted for such experimental work in that it permits quantitative determination of the influence of the disease upon the host;

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[2] Reference is made by number (*italic*) to "Literature cited," p. 85-86.

This observation may be repeatedly noted in infested soils, and it was with the idea of explaining this condition particularly that the investigation on the influence of environmental conditions upon the disease was begun in 1914.

Sorauer (*23*) in 1895 made some observations upon the rootrot on cyclamens, and concluded that heavy manuring, too abundant watering, and too high temperatures favored the attack by the parasite.

Peglion (*20*), who first reported the disease on tobacco in 1897, also believed that too much manure and water were controlling factors in producing the disease.

Campbell (*5*) believes excessive quantity of humus in the soil predisposes the tobacco plant to disease, and also that an acid condition of the soil weakens the plant and predisposes it to disease.

Buttaro (*4*), probably following the lead of other European authors, also writes that the disease on tobacco is favored by abundant organic matter, excessive humidity, and high temperatures.

Benincasa (*1*) concludes that in some years the disease appears only slightly or not at all, and states that its development is favored by too much organic matter, excessive watering, and generally damp weather. Benincasa, at about this time, began to study the relation of moisture and different kinds of soils to the development of the disease. He concluded at this time that porous soils give the best results and in 1911 he discussed the subject in more detail.

Cappelluti-Altomare (*6*) concluded that the disease could be checked by limiting the amount of watering and by not reducing too greatly the light and air supply of the seed beds. He also advises against sowing the seed too thickly.

Galloway (*11, pp. 174–178*) reporting on the wilt of violets, caused by *T. basicola*, advises against the use of decaying vegetable matter in the propagating beds. His statement that—

> plants affected may make a good growth in summer and show no evidence of trouble until September or October, when they will wilt more or less during the day and revive at night.

is especially pertinent to the investigations in this paper.

Clinton and Jenkins (*9*) suggest that excessive fertilization, soil reaction, and soil moisture, the latter in particular, may be important secondary factors determining the extent of the injury by *T. basicola*. They also state that the cold, wet weather of early spring helps along the trouble in the seed beds, particularly when they are not properly ventilated.

Clinton (*8*) is quite convinced that the character of the season, especially the moisture and possibly unusually cold wet spring weather, and the character of the soil and subsoil—fineness, liability to become water-soaked, drainage, amount of humus, especially in the shape of manure—have much to do with determining whether or not the fungus

does much damage. No definite experimental data are given, however, in support of these views. The following year (1908) drouth is said to have reduced the injury due to the disease.

Briggs (*3*) reported, upon evidence obtained from Connecticut soils that the fungus attacks are most severe on soils made alkaline by large applications of lime, ashes, or fertilizers containing carbonate of potash, and that the alkaline condition in infested soils should be corrected by the use of acid fertilizers in order to obviate the damage by *T. basicola*. This advice was received favorably by both practical growers and scientists, and many recommendations were based upon it.

Gilbert (*12*) concludes that an abundance of humus, a considerable percentage of clay, high fertilization either with chemicals or manure (expecially nitrogenous fertilizers), excessive water, and high temperatures favor the disease. In an experiment to determine the influence of the amount of watering he found that excessive water increased the disease, although 62 per cent of the plants in the scantily watered beds were diseased. He also compared the yield as a result of transplanting diseased and healthy plants in the field, using a "Havana Broadleaf" variety. He obtained as good yield from the diseased plants as from the healthy ones.

Whetzel and Osner (*27*) recommended acid-phosphate fertilization for the control of *T. basicola* which causes fiber-rot on ginseng.

Benincasa (*2*) reporting on results obtained in comparing different "soils" for growing tobacco plants, recommends sand or "*pozzolano*" a volcanic ash for this purpose, since favorable conditions for disease are said to be absent in these . He also states that *T. basicola* is a weak parasite under certain conditions.

Martinazzoli (*18*), however, reported that he obtained *T. basicola* from beds where *pozzolano* was used, infection probably having come from soil.

Massee (*19*) concludes that *T. basicola* can not infect host plants in pure sand, since the fungus is able to infect only in the presence of organic matter which will permit the mycelium to exist for some time as a saprophyte.

Chittenden (*7*) had difficulty in obtaining infection with *T. basicola* until overwatering of the soil was practiced.

Rosenbaum (*22*) believes that such external conditions as excessive water, lack of aeration, and heavy manuring favor infection.

Reddick (*21*) reported unsatisfactory results for the control of Thielavia of violets by acidifying the soil with acid phosphate as recommended by Briggs. Stable manure apparently did not act deleteriously on infested soil. The experiments were not carried far enough, however, to be entirely conclusive.

The present writer (*14*), as a result of field observation, also believed soil moisture to be the main controlling factor in determining the severity of the disease.

GENERAL CONSIDERATION OF FACTORS CONCERNED

It is evident at the outset that any attempt at a separate analysis of each factor concerned in disease occurrence is practically impossible. Varying one single factor of the environment to the total exclusion of variability in all others is an ideal to be kept in mind in experimental work of the nature to be described. Failure to reach this ideal in practice, however, need not necessarily reduce the value of the result, provided the effect of other variables on such a result is properly considered. As an illustration of a complication of factors of this sort, there may be cited the maintenance of two pots of soil at two different temperatures, say 30° and 10° C., respectively, in order to compare the effects of these temperatures on the occurrence of *T. basicola* on the roots of tobacco. By means of proper controls in uninfested soil the influence of many factors involved may be eliminated, but it does not seem possible to separate clearly the factor of soil temperature from that of soil moisture. The soil and plants at 30° will require several times as much water as will the soil and plants at 10° because of the increased evaporation and transpiration at the higher temperature. The correct replacement of this water for maintaining like moisture relations for the host and parasite is uncertain no matter how frequently and carefully it may be done either by weight or by the use of an auto-irrigator. If, however, the moisture relations have previously been studied and the range of the effects to be expected from this factor are known, it may be possible to carry on soil-temperature studies with only moderate attention to the moisture relations.

A study of the factors concerned in the development of the Thielavia rootrot has served to bring out clearly the fact that all the factors concerned are inseparably connected with one another, and that the amount of disease occurring is the product of a number of plus and minus factors, but that, nevertheless, in an analytical study of this nature, it is possible to arrive at the relative importance of these various influences.

It is important, furthermore, that not only the true environmental factors be taken into account, but also that such inheritable factors as the relative degree of susceptibility of the host plants used and the virulence of the parasite concerned receive proper consideration. In addition, the amount of infection and the time of its occurrence may greatly modify the results both as regards the readiness with which the host may become infected and the effect of a "mass action" upon the measurable end result of disease.

The purely environmental conditions to which the roots of the host or the parasite harbored by the soil are subjected may be conveniently considered under the following subjects: (1) Amount of infestation present; (2) percentage of moisture; (3) temperature; (4) soil reaction; (5) physical

structure of the soil, including the relative amount, the state of the vegetable matter, and the size of the mineral particles; (6) available chemical fertility; (7) state of cultivation as regards compactness and aeration.

To the soil physicist, chemist, and biologist it will appear that the entire scope of soil science may be concerned in the production of disease in the roots of plants, and such seems to be the case. On consulting the branches of soil science it is at the same time both encouraging and discouraging to find many of these factors influenced by a number of other interrelated factors under normal conditions. For instance, soil temperature as such can not be thought of without also considering the influences of the air temperature, specific heat, moisture content, exposure, and color of the soil upon such temperature. With soil moisture it becomes essential to regard moisture-holding capacities, rainfall, drainage, cultivation, humidity, and temperature; or, in the case of soil fertility, to consider along with the natural fertility, its cropping history, applied fertility, and various other modifying factors.

With these things in mind, however, it has become increasingly possible to account for, if not to explain fully, seeming contradictions and lack of accord with established principles of infection which have come under the writer's attention during the past five years in the case of the rootrot of tobacco. The occurrence and economic importance of the disease in one State and not in another, on one farm and not on the neighboring farm, or on the hilltop in one field and in the low spots of another, as well as the total failure of a crop in a field one year followed by a complete success the following year, or the change of crop prospects from failure to 100 per cent yield within the period of two weeks, are all more or less subject to scientific interpretation from this viewpoint.

With respect to those factors, aside from environmental conditions which may influence experimental results, it should be said that as far as evidence from literature, or as far as the observation of the writer is concerned, there is nothing to indicate that specialized races of *T. basicola* occur, or that the fungus varies in any way in virulence owing to differences in strain or age of cultures. It may be said with considerable certainty, therefore, that we are dealing with a relatively constant organism as to pathogenicity. With respect to host differences it has been shown (*13*, *14*) that very decided differences in susceptibility in host plants, and in varieties and strains of tobacco occur. By using pure strains of seed experimental error from this source may be eliminated. It should be remembered, however, as will be shown in the data here presented, that because of these differences in susceptibility the critical points in disease occurrence and severity may be shifted in one direction or another to some extent, a fact which makes it important that the susceptibility of the variety used for experimental work be taken into account in any interpretation of results.

AMOUNT OF INFESTATION

With diseases in which the injury from infection is confined to relatively small local areas on the host, it is to be expected that the amount of damage done is in some measure proportional to the number of infected areas which occur. Although it is evident that under the most favorable conditions, *T. basicola* may spread a considerable distance over a single root from one source of infection, more commonly the diseased portions are confined to areas of from 1 to 5 mm. In either case it is evident that relatively few infections may cause no appreciable stunting in growth, where a larger number of infections in proportion to the size of the root system may produce a rapid check in growth, owing largely to the mechanical reduction of the feeding area, and possibly also in some degree to the formation by the fungus of toxic substances injurious to normal metabolism. The latter possibility appears not to hold, however, since the host is rarely killed by the parasite. In fact, the plant appears to receive a stimulus toward the formation of new roots to replace those lost by disease, and in many instances the youngest leaves of infected plants possess a deeper green color than healthy plants. The new roots are at a greater disadvantage than the original ones, if they are formed at or near the region of disease, since frequently they are obliged to pass through small local areas of high infestation before reaching the deeper layers of soil.

It is a well-recognized fact that infestation increases under field conditions at an enormous rate once it is present or introduced to a soil which is being cropped to a highly susceptible host. This is illustrated in the practice in certain tobacco-growing areas of growing only one or two crops of tobacco and then laying the land aside for other crops for a long time. That this practice has been empirically developed, in the Burley section at least, as a result of the rootrot, seems unquestionable, in view of some unpublished results obtained in that section during the last three years.

The influence of the amount of infestation on the amount of disease might be illustrated in a number of ways. The simplest method appeared to be the mixing of varying quantities of infested and uninfested soil and transplanting into this mixture a susceptible variety of tobacco. The soil selected for this purpose was from the old tobacco field on the Station farm at Madison, on which tobacco had been grown continuously for 10 to 12 years, together with soil of the same type from a neighboring plot located not more than 2 rods away, but never having been used for growing tobacco. The fertility of the two soils naturally would not be the same, but the soil from the tobacco field because of heavy applications of fertilizers was the more fertile of the two from a chemical standpoint. The soils, after having been thoroughly screened, were weighed out and mixed in the proportion shown in Table I. Two sepa-

rate experiments of this nature were carried out. In a third experiment, steam-sterilized tobacco-field soil was used for mixing with the untreated tobacco soil in the same way as before. A gradual falling off in growth will be noted with the increase of amount of infested soil (Pl. 1, I). In the case of No. 4, in experiment 3, the lowered yield, as compared with all infested soil is no doubt due to the frequently observed fact that reinfested sterilized soil favors the growth of fungi and consequently the increased development of disease in the soil. In a mixture of three-fourths infested and one-fourth steamed soil the balance of infestation and conditions apparently was such as to cause greater infection than in all-infested soil.

TABLE I.—*Influence of amount of infestation of soil by Thielavia basicola on the yield of tobacco*

Pot No.	Soil mixture.		Average air-dry weight of duplicates.		
	Infested.	Uninfested.	Experiment 1.	Experiment 2.	Experiment 3.[a]
			Gm.	*Gm.*	*Gm.*
1	None	All	3.25	5.37	6.93
2	One-fourth	Three-fourths	1.65	3.66	4.20
3	One-half	One-half	1.40	2.73	2.65
4	Three-fourths	One-fourth	.70	2.64	[a].51
5	All	None	.01	1.32	1.33

[a] Steam-sterilized soil was used as uninfested soil in Experiment 3. The low yield in pot 4 was probably due to steamed infested soil favoring disease more than unsteamed infested soil.

The results obtained in Table I are considered to be due only to the fact that more spores exist per unit of soil in the higher proportion of infested soil, and therefore the roots are more likely to come in contact with infecting material. This fact has an important bearing upon results obtained in a study of environmental conditions. However, since any deleterious or beneficial action to which the parasite is subjected may merely reduce or increase the amount of infestation, the results will be proportional in some measure to the amount of infestation present.

INFLUENCE OF MOISTURE CONTENT OF SOIL

A review of the literature concerning factors influencing the severity of the rootrot of tobacco shows that soil water has been considered the primary limiting factor by practically all observers and investigators of this disease. Most of the conclusions drawn in regard to this, however, have been based on observations in the greenhouse or in plant beds, where artificial watering is resorted to and where it is relatively common in many instances to overwater the soil. A study of the disease under field conditions with reference to moisture, though equally indefinite and inconclusive owing to the number of other variable factors, is at any rate convincing that an oversupply of moisture is not necessary for heavy infection and severe attacks by the parasite.

In order to get more accurate evidence on the influence of soil moisture, a series of pot experiments, with the moisture supply controlled as closely as possible by weight, were carried out.

Several difficulties, not readily overcome, exist in such an experiment, the principal one being that it is practically impossible to maintain a uniform moisture content throughout the soil. No doubt the use of Livingston auto-irrigators would have made possible more uniform results, but these were not available at the time. Two-gallon crocks, perforated at the base for drainage and holding about 10 kgm. of soil, were used. The naturally infested soil from the old tobacco field on the Station Farm was used. After a large quantity of this soil had been dried, thoroughly mixed, and screened, its moisture content and water-holding capacity were determined in the ordinary manner. Ten kgm. of the soil were then placed in each of twenty 2-gallon crocks. The soil in 8 of these crocks was sterilized by steam at about 100° C. for the purpose of destroying all the *T. basicola* present in order to provide disease-free controls in the experiments. The water relations, as well as the food relations, were, of course, changed in some degree by the sterilization, and an absolute comparison between the sterilized and infested series was therefore not permissible, although it is believed that the results are not altered appreciably by this fact.

The crocks of soil were then divided into four series, each containing three infested and two uninfested crocks of soil. Two glass tubes, ½ inch in diameter, one being inserted to a depth of 2 inches and the other to a depth of 6 inches, were placed in each crock for the purpose of permitting a more uniform distribution of water in the soil. Of the four series, one was now made up to one-fourth its full water-holding capacity, and the others to one-half, three-fourths, and full water-holding capacity. After the water had been allowed to distribute itself fairly evenly, one plant of the White Burley variety grown in sterilized soil was transplanted to each of 20 crocks. The loss of moisture from the crocks was very slow when the plants were small, especially during the winter in the greenhouse. Usually it was not necessary to make the pots up to the required weights oftener than once every three days, but later in the tests daily attention was usually necessary. In an experiment begun on February 13, 1917, with the White Burley variety, it was noted at the end of one week that in the infested series the plants at one-fourth saturation wilted during days of high transpiration and showed the poorest growth. The plants at three-fourths saturation got the best start, while those at full saturation were already yellowing and apparently diseased, since no such condition was observed in the sterile controls. On March 5 the conditions were about the same in relative growth except that the diseased condition of the plants at full saturation in infested soil was greatly increased, and the controls in sterilized soils were now beginning to forge rapidly ahead of those in infested soil. On March 14 it seemed quite

evident that in uninfested soil the optimum moisture content of this soil type for the growth of tobacco lay close to three-fourths saturation and that full saturation was more favorable than the one-half and one-fourth saturation. Although still no great differences existed between the infested and uninfested soils at one-fourth, one-half, and three-fourths saturation, the plants in the uninfested soil at full saturation were about 10 times as large as those in the infested soil at the same saturation.

The data taken upon the growth of the plants in these experiments are recorded for the most part as the total leaf area of each plant taken at intervals of about one week. These determinations were made by placing the leaves over a standard leaf-area chart on which areas for varying sizes and shapes of leaves had been previously determined with a planimeter. These areas, expressed in square inches, were determined at the end of this experiment, on March 28, and are given under experiment 2, Table II. The results appear to justify the conclusion that a fairly constant ratio exists between the growth on infested and uninfested soil at the three lower saturations. This ratio is approximately 1 to 3½. On the soils at full saturation, however, the ratio of growth on infested soil to that on uninfested soil is about 1 to 40. The evidence from this experiment therefore shows that a very considerable amount of disease can occur in a relatively very dry soil and that it does not appear to be proportionately increased in a relatively moist soil, but that a wet or saturated soil, which still permits a good growth of tobacco when uninfested, causes a rapid decrease in yield when infested with *T. basicola*.

TABLE 2.—*Influence of the moisture content of the soil on the amount of tobacco rootrot*

Saturation.	Approximate percentage of moisture.	Experiment 2: Average leaf area (square inches).		Experiment 4: Average air-dry weight (gm.).	
		Uninfested soil.	Infested soil.	Uninfested soil.	Infested soil.
One-fourth	7.7	34	9	2.0	1.5
One-half	15.5	81	22	9.8	4.5
Three-fourths	23.3	301	89	19.7	5.4
Full	31.0	239	7	9.3	1.1

In a following experiment, which was conducted on the same soil in the greenhouse, the above results were practically duplicated so that the data will not be presented here. A third experiment was conducted during the growing season in a shelter out of doors, permitting atmospheric relations more nearly normal than those occurring in the greenhouse during the winter season. The experiment was run in the same manner as the foregoing one, except that a change of soil was made, another batch from the same source was used in order to avoid nematode injury. The crocks were set to White Burley on July 5. On July 12 all the plants appeared to have a good start except those at one-fourth

saturation, which usually wilted during the daytime owing to lack of moisture. By July 21 marked signs of heavy infection of all the plants in the infested soils was shown by reduced growth and yellowing of the lower leaves; this condition was most marked at full saturation. The uninfested soil showed the optimum growth at three-fourths saturation. On August 14 the experiment was discontinued, the plants photographed (Pl. 1, II–III), and then cut and dried. The air-dry weights are given in Table II under experiment 4.

While the results of this experiment as shown by air-dry weight in comparison with the leaf area given in experiment 2 do not conform in all details with those of experiment 2, they are believed to agree in general in that the greatest amount of injury from disease resulted in the saturated soil; the ratio of the yield on infested soil to that on uninfested soil was again considerably greater than in the other cases.

It is realized that further experimental evidence could be profitably obtained as to the relation of moisture to the disease. The difficulties already referred to, however, together with complication of other factors such as temperature, and some of the more obscure factors such as aeration and compactness of the soil, have rather discouraged further tests until more accurate technic can be devised. It is certain, however, that T. bascicola has a wide range of action as regards actual percentage of moisture present in the soil. It has been found, for instance, that in water culture containing a spore suspension, good infection occurs on roots and that it will occur in soils too dry to permit anything like normal growth of tobacco. Whether there is a direct increase in amount of infection and severity of the disease with percentage increase of moisture in the soil may not be exactly clear from the data here presented. From the majority of the data obtained, however, some of which is not given here, it seems fairly certain that such direct proportionality does not exist, but that a fairly constant relation is maintained in soils with moisture content ranging from those sufficient only for poor plant growth to those approaching saturation, followed by a very rapid increase of disease from this latter point up to full saturation.

At any rate it may be said that poorly drained infested soils which are likely to remain saturated for a period of three or four days, or any infested soil kept near saturation for a period of days due to excessive rainfall will undoubtedly show higher infection than well drained soils or soils not affected by an excessively wet season. On the other hand it appears that, as a rule, soil moisture is not an important controlling factor in the prevalence of the rootrot of tobacco. Relatively dry or relatively wet seasons, in so far as they affect soil moisture alone, are not especially to be feared nor to be relied upon for holding the disease in check. For the same reason a careful check has not been kept upon the moisture content of the soils in the various experiments described

in this paper with other environmental conditions. The soils have been kept as nearly as possible uniformly watered, never approaching saturation for any considerable period of time and never relatively dry, so that it is not believed that the results have been vitiated by this factor, though it is admitted that they may have been responsible for many of the individual variations in results always occurring in experiments of this sort, and which is planned to be overcome by mass of data rather than by the most careful attention to a single experiment.

If the above-ground symptoms of the rootrot are considered, it is at once realized that the reduction of the water supply is probably the most important one, partly as a result of reduced food supply to the plant brought about by the gradual but effective depletion of the root system. It is therefore usually quite impossible to judge from the above-ground portion of plants alone as to whether lack of available soil moisture or lack of roots or both are responsible for a reduced yield. It is only when growers obtain a greatly reduced yield on land known to be in a high state of fertility that they begin to suspect other troubles. It is said with confidence, therefore, that fully nine-tenths of the damage by the rootrot is attributed by the growers either to a deficiency or to an excess of soil moisture. Sixteen moisture determinations of the field plots (the same soil as used in the pot experiments) at about 3-day intervals between June 19 and August 6 in the summer of 1917 showed a range of moisture content from 25.3 per cent on June 25 to 16.2 per cent on August 2. These determinations showed that during the entire season the moisture content was practically between the limits of one-half to three-fourths saturation. In these plots White Burley tobacco made no growth whatever during this time because of *T. basicola*, and Connecticut Havana made only half a crop. It is clear that the moisture content was not excessive for the best growth of tobacco, and yet the parasite was almost at its maximum of activity.

INFLUENCE OF SOIL REACTION ON ROOTROT

The reaction of the soil has been considered to play a part in the severity of parasitism in practically all plant diseases having their origin in the soil. The reasons for these rather widespread calculations are perhaps manifold. Among the earliest chemical agents applied to soil with the hope of checking plant pests was lime, and experiments too numerous to mention here have since been conducted with it in the hope of checking the diseases and insects attacking plants. Where lime has proved efficacious, however, pathologists have considered it both as influential as a sterilizing agent against the parasite and as a neutralizer of soil acidity favorable to parasitic action. The beneficial action of lime to the growth of green plants and to bacterial activity in soils has also no doubt served to stimulate its use in phytopathological problems. No advantage is to be gained by reviewing the rather extensive study of

the value of lime in the control of numerous plant parasites harbored in the soil because the results obtained depend altogether upon the disease concerned. Experiments in its use have been most complete in relation to the control of potato-scab, clubroot of crucifers, and nematodes. Limed soils favor scab, whereas clubroot and nematode injury are much reduced by its use.

With the appearance of a paper by Briggs (*3*), based on field experiments in Connecticut, a great deal of interest was revived on the influence of soil reaction on plant diseases having their origin in the soil. Briggs concluded briefly that materials applied to the soil which tended to make it alkaline in reaction favored Thielavia-rootrot of tobacco, whereas materials applied which made the soil acid reduced the disease. The actual change in soil reaction apparently was not determined. On the basis of these results the use of acid fertilizers came to be recommended in both scientific and popular literature and the use of lime cautioned against. Considerable experimental work also was undertaken at various places with this and other diseases, some of which apparently verified the results of Briggs, while others showed no favorable results. Thus, the problem has remained in a more or less uncertain state. Clearly it is not one which is easily solved. Changing the reaction of the soil from acidity to alkalinity, and especially from alkalinity to acidity by the application of different chemicals, is open to many difficulties not clearly analyzable. The problem of soil reaction as influencing disease presents two aspects: First, to determine the actual influence of the reaction of the soil medium upon the disease; and second, to determine in how far this influence may be utilized in a practical manner by actually changing the reaction of large areas of soil to a sufficient degree to modify the severity of the disease. The latter problem is complicated by a number of factors, the most evident of which is the naturally or normally attained reaction of the soil, since this must have considerable bearing upon the amount of acid-producing materials which must be applied to get the desired result. Aside from the final influence of such treatment on the soil itself, in a system of economical and permanent agriculture a more discouraging feature of the problem is that from the standpoint of the disease, for, as will be shown, many other factors must be taken into consideration, such as the susceptibility of the variety of tobacco grown, the amount of infestation, and the temperature of the soil. By varying these factors markedly different results may be secured on the influence of soil reaction in relation to disease.

The experiments carried out in the investigation presented here were of two kinds, pot tests and field plot tests. The former were carried on for the most part in the greenhouse during the winter season and the latter on an old heavily infested tobacco field on the Station farm at Madison.

POT EXPERIMENTS

As has already been suggested, it is especially difficult to render an alkaline soil acid in various degrees by the application of a theoretical quantity of an acid or acid salt. The alteration produced in the soil by either treatment is likely to alter it so fundamentally that comparison with another soil treated in a dissimilar manner tends to complicate the results to an unnecessary degree. What seems to be a considerably better plan is to select a naturally highly acid soil and to change its acidity to various degrees of alkalinity by the application of the theoretically correct quantities of lime. Accordingly, this plan was followed.

The soil selected was a very acid Sparta sand from a field at Lavalle, Wis. A total acidity determination of this soil by the Truog method (*26*) showed that its lime requirement was 9.38 tons per acre. The strength of acidity was found to be 108 on the basis of acetic acid at 1,000. The soil after being finely screened was placed into 2-gallon stoneware crocks, perforated at the base for drainage. Ten kgm. of soil were weighed into each of 36 crocks. These crocks were then divided into 9 sets of 4 crocks each. The calculated quantity of precipitated calcium hydroxid of the highest purity was thoroughly incorporated in the soil of each of the 4 crocks in each series with a view to reducing the acidity to fairly definite degrees. In Table III are given the quantities of calcium hydroxid applied, together with the determinations of total acidity by the Truog method made several months later, when it was considered that the full effect of the treatment on the soil had occurred.

TABLE III.—*Influence of soil reaction on development of rootrot*

Series.	Quantity of lime added to 10 kilograms of soil.	Lime requirement per acre.	Average air-dry weight.						
			Experiment I (White Burley).			Experiment II (Connecticut Havana).		Experiment III (Maryland Broadleaf).[a]	
			Infested soil.	Uninfested soil,	Amount of infection on roots (infested soil series).	Infested soil.	Uninfested soil.	Infested soil.	Uninfested soil.
	Gm.	*Tons.*	*Gm.*	*Gm.*		*Gm.*	*Gm.*	*Gm.*	*Gm.*
1.....	0.0	9.38	2.46	2.72	Very slight.....	6.35	6.45	0.11	6.80
2.....	17.80	7.19	1.09	4.09	Considerable.....	2.65	5.95	.11	5.00
3.....	35.60	4.60	.33	4.65	Heavy.....	2.22	5.82	.18	8.15
4.....	53.40	2.62	.17	4.40	.....do.....	.75	8.20	.14	8.67
5.....	71.25	.72	.68	1.49	.....do.....	2.06	5.87	.17	10.35
6.....	89.05	.....	.24	1.53	.....do.....	.57	7.12	.14	7.12
7.....	106.85	.....	.40	.79	.....do.....	1.99	6.90	.18	7.10
8.....	124.60	.....	.38	1.24	.....do.....	.43	6.59	.24	5.22
9.....	142.40	.....	.23	.73	.....do.....	.58	5.82	.16	4.90

[a] Heavy infestation.

Two pots of each series were inoculated with pure cultures of *T. basicola*, and young seedlings of the susceptible White Burley variety were transplanted into them. The first test of plant growth failed,

owing, probably in large measure, to poor infestation; and the second test was ruined by a heavy infection of nematodes. All the soils were then sterilized by steam, and two of each series again inoculated, this time by the application of equal quantities of chopped-up, air-dried, heavily infected roots which had been taken in the fall from the tobacco field. The pots were again set to young seedlings of White Burley from steamed soils. The infestation now proved to be good, but complications arose in the limed end especially, owing either to the influence of the lime itself or to the sterilization alone or more likely to the two combined. It seems most probable that the lime was concerned in rendering the phosphates unavailable to the plants, but, as was expected, this injury was probably not a factor in the following experiment. However, this test, which has been designated as experiment I, in Table III, produced some fairly striking results in the infested series.

It was evident during the early growth of the plants that those in the infested soil of highest acidity were making considerably better growth than those at the alkaline end. It also appeared early that the line of demarcation between heavy infection and reduced infection in this series did not lie near the point of neutrality but well into the acid end and so the soil requiring 4.6 tons of lime per acre was as productive of disease as any at the alkaline end. Growth of all the plants was slow as the soil was not very fertile and the light poor. The plants, therefore, were harvested when still relatively far removed from the blossoming stage. They were dried at about 80° C. for several days, and then allowed to come to air-dry weight. The data given under experiment I, Table III, sufficiently illustrates the results obtained. The roots were carefully removed and examined for lesions of the disease, and, as was expected, these were correlated with the growth of the plants. In the soil requiring 9.38 tons lime per acre only a most careful search revealed any *T. basicola* at all. In the next lowest series (7.19 tons per acre), although considerable disease was present, plainly its development was markedly checked, whereas in all the series below this practically no uninfected portions of roots existed.

The crocks were now replanted with Connecticut Havana tobacco, a semi-resistant type. These were allowed to grow for about 50 days before being harvested. The air-dry weights are given under experiment II in Table III. It will be noted that the soil at the alkaline end practically recovered from the injurious properties previously described in the control series. In the infested series it may be noted that the point of effectiveness of the acid reaction in reducing the disease shifted to a somewhat lower degree of acidity, undoubtedly due to the greater resistance of the variety (Pl. 4, I–II). For some unexplainable reason the plant in one of the pots of series 5 and also one in series 7 failed to become as seriously infected as those in the neighboring pots. The increased yields in series 5 and 7, however, are not considered as inter-

fering with the general conclusions to be drawn from the experiment, and the writers have again shown that the highest acidity practically eliminated damage from rootrot, but that heavy infection still occurred in fairly acid soil.

It was believed that the abnormal behavior of the two soils in series 5 and 7 might be due to reduced infestation. All the crocks, therefore, were resterilized and the two of each series reinfested with 100 cc. of a fairly heavy suspension of endoconidia of *T. basicola* in water. This was thoroughly incorporated throughout the 10 kgm. of soil of each crock. Maryland Broadleaf tobacco, a variety almost as susceptible as White Burley, was then transplanted into them soon after inoculation. The results of 65 days of growth are shown in the air-dry weight under experiment III in Table III. A heavy infestation apparently reduced the efficacy of the acid soils to nothing, at least in the presence of a susceptible variety. The disease appeared, in fact, more virulent in the most acid soil.

The soils were now again replanted to Connecticut Havana, the semi-resistant type. The actual amount of infestation was also probably somewhat reduced, as many of the spores originally introduced must have spent themselves, although it is probable that the fungus was living in the soil as a saprophyte. Results similar to those obtained in experiment I were now secured, indicating that partial recovery from infestation had occurred in series 1 and 2.

The question arises as to just what effect soil reaction has upon the occurrence of the disease. High acidity may increase the resistance of the host plant; or it may act deleteriously upon the germination of the spores or the growth of the parasite. If we assume that acidity increases the acidity of the cell sap and, hence, the resistance to disease, as suggested by Comes (*10*) for cereal diseases, we have a working hypothesis which is, however, difficult to establish definitely. It has been shown that *T. basicola* (as do most fungi) grows best on an acid medium (about 1 per cent). Water extracts of the soils from the various series were made which represented approximately the concentration of the soil solutions. Germination tests of endoconidia in these extracts showed better germination in the acid end than in the alkaline end. Other soil extracts tubed with agar showed better growth of *T. basicola* at the acid than at the alkaline end. Although the acidity from the higher acid series was such as would not permit the growth of bacteria, yet tests of this nature probably fall short of resembling the actual acidity in the soil. The results in experiment III show, of course, that spore germination and fungus growth are not completely inhibited by an acidity requiring 9 to 10 tons of lime per acre. The writer is inclined to believe, however, that the beneficial action of soil acidity in reducing infection by *T. basicola* is due to a gradual depressing effect upon the fungus.

FIELD EXPERIMENTS WITH SOIL REACTION

The field plots were located on the Experiment Station farm at Madison on a tobacco field which had grown 10 and possibly 12 successive crops of tobacco, and on a neighboring field which had previously grown only 1 crop of tobacco. The infested field had for three or four years previous to this experiment shown itself to be heavily infested and would grow only half a crop of Connecticut Havana tobacco, while White Burley would make no growth whatever on this soil, especially during relatively cool growing seasons. This soil had had heavy annual applications of barnyard manure and was in a good state of fertility as shown by corn and cereals growing in adjacent plots. The soil reaction at the beginning of the experiments was practically neutral.

A control field across the road on uninfested soil was started for a double purpose. In the first place it made it possible to check up the beneficial or injurious action of the fertilizer and lime applied, aside from infection from disease. In the second place it has been considered that, although the application of acid fertilizer might not remedy the condition in a badly infested field, it might serve to hold down the rate of infestation of new soil to a considerable degree. This soil is equally as fertile as the infested field, but shows a slightly greater degree of natural acidity, being classed as slightly acid according to the Truog color chart. Since it was found in the pot experiments that a very considerable range of reaction was required to make any appreciable difference in amount of infection by *T. basicola*, it was decided to use the more simple though fairly accurate comparative test of Truog (*26*) with lead-acetate paper. The reference to the degree of acidity, therefore, will be based on the standard color chart accompanying the description of this test.

The plots used were one-fortieth acre in size. The applications were made in two different amounts, a heavy application and a light application, also referred to as a full application and a half application, respectively. The original plans of the experiment called for the use of alkaline fertilizers—that is, potassium carbonate, basic slag, and nitrate of soda, with equivalent amounts of sulphate of potash, acid phosphate, and sulphate of ammonia for the acid fertilizers. On account of the apparent impossibility of obtaining all of the alkaline fertilizers, it was decided to use the acid fertilizers and heavy applications of lime to produce the alkaline condition. The rates of applications, in pounds per acre, then, are as follows:

Acid plots.	Full amount.	Half amount.
Sulphate of ammonia	1,200	600
Sulphate of potash	1,200	600
Acid phosphate	2,400	1,200
Alkaline plots.		
Slaked lime	12,000	6,000
Sulphate of ammonia	1,200	600
Sulphate of potash	1,200	600
Acid phosphate	2,400	1,200

The first application was made on June 18, 1917. The applications were made by hand, disked, and harrowed in. The lime was previously allowed to slake in the field. Connecticut Havana tobacco was transplanted on all the plots on June 27. Acidity determinations made one month after the applications showed slightly increased acidity for the acid plots and slightly decreased acidity for the alkaline plots. Determinations unfortunately were not made at the end of the season, but the tests for the following year served to indicate that, although the changes were not great in degree, they were decidedly effective in bringing about a marked change in reaction between the alkaline and acid plots.

In the first year's tests the acid plots in the infested soil showed up decidedly the poorest throughout most of the growing period, while the heavily limed plot was decidedly the best in the series. On the new field the fertilizers both with and without lime gave somewhat better results than the controls. So far as can be judged by the results, the acid fertilizers were not injurious to the crop on this soil although it is possible, of course, that these materials might have had some direct injurious action on the tobacco in the case of the infested soil. Apparently, such a condition did not occur on the uninfested soil, nor on the infested soil when the plots treated in the same manner were limed.

The plots were harvested and cured separately; the yield of cured leaves for 1917 are given in Table IV.

TABLE IV.—*Yield of tobacco on soil with acid fertilizers, with and without lime, 1917–18*

Treatment.	Plot.	Application on $\frac{1}{10}$ acre.		Yield of cured leaf on $\frac{1}{10}$ acre (pounds).				
				1917.		1918.		
		Acid fertilizers.	Lime.	Infested soil.	Uninfested soil.	Infested soil.[a]	Uninfested soil.[a]	Infested soil.
			Lbs.					
Alkaline	A	Sulphate of ammonia, 30 pounds. Sulphate of potash, 30 pounds. Acid phosphate, 60 pounds.	300	38.5	44.5	31.3	34.0	31.3
Do	B	Sulphate of ammonia, 15 pounds. Sulphate of potash, 15 pounds. Acid phosphate, 30 pounds.	150	26.0	40.0	29.0	39.5	31.0
Control	A	None	None.	24.0	33.5	38.5	39.0	30.0
Do	B	None	None.	22.0	33.0	36.0	.....	28.0
Acid	A	Sulphate of ammonia, 30 pounds. Sulphate of potash, 30 pounds. Acid phosphate, 60 pounds.	None.	15.0	44.5	30.5	41.5	30.5
Do	B	Sulphate of ammonia, 15 pounds. Sulphate of potash, 15 pounds. Acid phosphate, 30 pounds.	None.	15.5	38.5	29.3	41.5	29.3

[a] 1917 series. These plots received a second application of same amounts in 1918 and had, therefore, the residual effect of the 1917 applications.

The results were sufficiently interesting to warrant repetition the following season (1918) on a slightly increased scale. All the plots were again given an additional application, the same amounts as in 1917 being used. In the infested field six plots were added, these being given

the same treatment as the others, the essential difference being that these did not have the residual effects of the previous season's applications and would therefore be more directly comparable with the plots in 1917.

The applications of lime were made on May 21 and fertilizers applied on June 3. On June 12 all the plots were planted to Connecticut Havana tobacco. On June 21 samples of soil were taken from each of the plots and tested for reaction in the ordinary manner.

In the infested soil the control plots showed very slight acidity. The acid plot of last year (full amount) showed medium to strong acidity; the half-amount plot showed slight acidity. In the same way the plots which had received the acid fertilization for the first time in 1918 showed nearly medium acidity for the full application and slight acidity for the half amount. None of the alkaline plots showed acidity and presumably were considerably below the neutral point, though this could not be shown by the test used. On the uninfested plots the change in acidity due to the application of the fertilizers were not so marked, probably only a slight change having been produced. The limed plots, however, showed no signs of acid reaction.

On June 18 it already appeared that on the full-limed plots the lime was acting injuriously upon the seedlings, both in the uninfested and in the infested soils. This may have been due in part to the fact that the lime was not well air slaked and was, hence, not thoroughly incorporated in the soil. The action of the lime was, therefore, probably toxic and probably vitiated the results, so far as lime was concerned, although the plants appeared to recover later in the season. The outstanding feature of the results in 1918 was again that the plots made acid with heavy applications of fertilizers under field conditions were on the average little or no better than the untreated plots (Table IV). In fact, the untreated plots of the 1917 series were considerably better than the acid-treated plots of 1917 or 1918, although the plots treated with acid fertilizers for the first time in 1918 were slightly better than their controls for this year. There is no question as to the extent of infection on this soil this season, since resistant and susceptible types planted at the same time behaved in the expected manner. In interpreting the results from the field plots it should be recognized that the tests are not exhaustive, and that on account of the complexity of the problem the conclusions drawn may not apply under all conditions. For Wisconsin conditions, however, it appears that the application of acid fertilizers to soils, alkaline or neutral in reaction, will not reduce infection by *T. basicola*.

SOIL TEMPERATURE AS A FACTOR IN ROOTROT

A review of the more important literature concerning the influence of soil temperature on diseases of plants and the importance of such studies has been presented by Jones (*16*). With respect to the influence of this factor on infection and severity of the rootrot of tobacco caused by

T. basicola practically nothing of a definite nature exists. Rather obscure statements that high temperatures favor the disease have been published by Gilbert (*12*), while, on the other hand, Clinton (*8*) states that possibly unusually cold, wet spring weather has something to do with determining whether or not the fungus does much damage. Galloway (*11, p. 174-178*) found that in the greenhouse the disease was apparently more severe on violets on the approach of fall than in summer, indicating a temperature relation. In Italy where very considerable observation has been made on the disease, it is agreed that weather conditions have much to do with its occurrence and severity. That such was the case in Wisconsin was evident during the first season of observation. The recovery of badly infected plants in large areas during the course of only two or three weeks led to the desire to study in more detail the environmental conditions bringing this about. It was at first suspected that the moisture relations were the all-important factor; but in connection with its study, temperature records of the soil under field conditions were taken beginning in the spring of 1915, and continued for the seasons of 1916, 1917, and 1918.

In the fall of 1916, following some interesting results by Tisdale (*25*) on the influence of soil temperature on flaxwilt (caused by *Fusarium lini*), the writers, under the advice and support of Dr. L. R. Jones, undertook to have a large tank (Pl. 2, I) constructed in which soil could be held fairly constant at several different temperatures. This tank has already been described and illustrated in some detail by Jones (*16*). Further detailed description of the mechanical part of the apparatus seems unnecessary, especially in view of the fact that improvements are being gradually made on these tanks which will no doubt necessitate further description of similar apparatus developed in the Department of Plant Pathology of the University of Wisconsin. It should be said, however, that by means of proper insulation of the compartments it has been possible to maintain a fairly constant temperature of water at any selected temperatures between approximately 5° and 40° C. This has been done by the inflow of cold water from the taps in the winter time and by heating the water to the higher temperatures with electric bulbs or with steam. The expense of automatic temperature regulation in a large number of chambers has discouraged the use of such apparatus up to the present time, but personal attention and regulation two and three times every 24 hours, in combination with good insulation, has been found to give results sufficiently accurate for most needs. It was found that although considerable ranges of temperature occurred at the extremes (below 15° and above 30°) the temperatures between 15° and 30° could be held quite constantly within 1 degree.

The soils used were placed in 1-gallon battery jars and set on boards suspended in the water in the tanks. Four jars could be placed in each compartment with displacement of only a relatively small amount of

water. Two jars in each compartment containing sterilized or uninfested soil were used as controls for plant growth alongside two jars containing infested soil. Naturally infested soil from the old tobacco field on the Station farm, previously referred to, was used in most of the experiments. After being given a good application of well-rotted manure, the soil was thoroughly mixed and screened before weighing equal quantities into the jars. The sterilized soil used in the earlier experiments was sterilized by steam to destroy the infestation by *T. basicola*. Considerable difficulty was experienced, however, as a result of the toxic action of the heated soils on plant growth at the lower temperatures, which interfered to some extent with the reliability and uniformity of the data obtained by leaf measurements.

In later experiments the employment of soil steamed two or three weeks previous to being used and allowed to stand in a moist condition at a fairly high room temperature reduced this action to a minimum. In still other tests formalin-sterilized soil was used with equal success, and in the final experiment another uninfested soil was used and artificial innoculation resorted to for the infested series.

The data taken in the earlier experiments were mostly in the form of measurements of leaf area in square inches as determined by a standard chart of various leaf sizes whose areas had previously been determined by the use of a solar planimeter. In later experiments air-dry weight determinations of the stalks and leaves were made.

The determination of the actual amount of disease on the roots is, of course, the final criterion for judgment, and in the last experiments it was found that with care the greater part of the roots could be washed out from the soil, examined for disease, dried, and weighed; these weights are closely correlated with growth aboveground, so that either the area of the leaves, weight of the leaves and stalks, or weight of the roots alone give a good index of the extent of the disease. A preliminary report of the results obtained has been given and an abstract published (*15*).

Eight separate experiments have now been made upon the influence of soil temperature on the extent of the root disease, four determinations being made in the winter and spring of 1917, and four during the fall and winter of 1917–18. The first experiments were made over a range of about 35° C., but, as these were found to be beyond the ranges of normal growth and infection, the later experiments usually included a temperature range of about 15°. Three of the experiments failed more or less to give uniform results; one due to nematode infection at the higher temperatures, another to toxic action of the heated soil, and a third to the accidental use of infected seedlings.

The procedure in each experiment consisted merely in filling the required number of jars with soil; one-half with uninfested and one-half with infested soil. Glass tubes 2½ inches long, were inserted into each jar to permit watering part of the soil at about half its total depth. After

being brought up to about three-fourths saturation, the jars were set in the tanks at the different temperatures and allowed to remain there for three to five days to permit the necessary changes of temperature. One young seedling of tobacco, usually the susceptible White Burley variety, was then transplanted into each jar. Subsequent attention then consisted only in taking the temperature records twice a day, in maintaining the proper temperature, and in watering the plants as required. In the first experiments, when the temperature range was determined, 12 different soil temperatures were run at one time; but in the latter experiments, when a closer approximation of the critical temperature was necessary, only 6 or 7 temperatures were used.

EXPERIMENT I.—Twelve temperatures were used, ranging from 7° to 40° C., and the white burley variety was transplanted into the jars. The plants in the uninfested or sterilized soil series grew best at temperatures of 29° and 31°. Practically no growth occurred below 13°, and again there was poor growth at 40°. From a physiological standpoint it was interesting to note that a marked effect upon the shape of the plants occurred especially at the higher temperatures. While the plants grew low and stocky with broad but rather pointed leaves at the optimum temperature for growth, the plants became tall and spindly, with short and rounded leaves at a temperature of about 36° to 40°.

In the infested soils at temperatures above 26° the plant growth appeared to be almost as good as that in uninfested soil (Pl. 2, I). At the temperatures 23°, 21°, 19°, and 17°, however, a very decided reduction in growth occurred as compared with the uninfested soils at the same temperature.

Upon removal of the roots from the infested soil series it was found that those at temperatures between 23° and 17° were heavily attacked by *T. basicola* and that slight infection occurred at 7°, while at 26° relatively few lesions occurred. The lesions were still less common at 29°, while at 31° only one lesion could be found. At the higher temperatures, 35° and approximately 40°, no signs of Thielavia infection were found.

EXPERIMENT II.—In this experiment the temperature range was 9° to 40° C. The toxic action of the heated soils at temperatures of 17° to 25° became quite marked early in the experiment, and no doubt affected the results. The total leaf area of each plant was determined at four different times during the course of the experiment. The results lack uniformity, however, owing to the toxic action of the heated soils. The infested soils gave the best growth at 35°, with an average of 251 square inches, as compared with 289 square inches for the sterilized soil at the same temperature. The poorest growth was at 19°, where a leaf area of only 19.2 square inches was obtained in the infested soil, as compared with 206.4 square inches in the sterilized soil. While the disease was quite marked at 24.5°, 71 square inches on infested soil as against 205 square inches on sterilized soil, decided indication of reduced severity again appeared at 26°, 169

square inches in infested soil as against 203 square inches on sterile soil. Examination of the roots in the infested series showed a relatively reduced amount of infection at 9° and 13°, heavy infection between 17° and 24.5°, much less infection again at 26° and 29°, and no infection at 31°, 35°, and 40°.

The results of Experiment II are in accord with the results of Experiment I, and apparently show in addition that the optimum temperature for the disease lies around 19° and 21°, although heavy infection still occurs as high as 24.5°.

EXPERIMENT III.—The same soil was used as in Experiments I and II. This soil was now so heavily infested with nematodes at the higher temperatures that the results with *T. basicola* were vitiated, and no data were taken.

EXPERIMENT IV.—New soil from the same infested field was used in this experiment, the proper care being taken to sterilize thoroughly the battery jars before filling them with soil. To reduce the harmful effect of the sterilized soils, the pots, after being filled, were allowed to stand moist for a week at room temperature before being placed in the tanks. In this experiment only seven different temperatures were used, which permitted the use of four jars of infested soil and four controls at each of five temperatures, but only two of each at the extremes. The final results are given in Table V. It may be again noted that the greatest amount of injury from disease occurred at the temperatures from 19° to 22° C., less occurred at 24° to 25°, while at 26° to 27° the injury was much reduced.

TABLE V.—*Influence of soil temperature on development of rootrot*

Temperature.	Average leaf area (in square inches) of duplicates.			
	Experiment III.		Experiment IV.	
	Uninfested soil.	Infested soil.	Uninfested soil.	Infested soil.
° *C.*				
13–15	63.3	28.5	71.8	41.7
15–17	106.7	24.9	87.4	13.0
19–20	206.4	19.2	137.6	9.5
21–22	212.3	26.7	304.2	21.4
24–25	205.1	71.1	430.9	197.6
26–27	202.7	168.7	306.1	280.4
30–31	256.4	243.6	339.3	327.1

EXPERIMENT V.—This experiment was largely a failure, owing to the use of plants that apparently were slightly infected by *T. basicola* and also by nematodes. No plants from sterilized soil were available at the time. The results were interesting, however, in that an examination of the roots showed that at the lower temperatures—that is, those favorable for infection—the heaviest infection occurred in the sterilized soil. This

is in line with the frequently observed fact that sterilized soil reinfested is a very favorable medium for the progress of disease. At 31° to 32° C. a trace of infection was found in the sterilized soil, but no infection occurred in the naturally infested soil. Nematode injury was most serious at the higher temperatures.

EXPERIMENT VI.—Six different temperatures ranging between 17° and 32° C. were used. The best growth of the controls in uninfested soil occurred at 31° to 32°, and the poorest at 17° to 18°. The best growth in the infested series was also obtained from 31° to 32°, which was practically equal to that of the controls. Almost equally good growth occurred at 28° to 29°, but at lower temperatures the results were again unfortunately interfered with by the toxic action of sterilized soil, which, though it had been treated for the purpose of reducing the toxicity, had not apparently sufficiently reduced the toxicity. Examination of the roots, however, which were carefully washed out, yielded results in line with the previous experiments.

EXPERIMENT VII.—In this experiment the soil in the uninfested series was sterilized with a 1 to 50 formalin drench three weeks prior to its use, in order to avoid further interference by the toxic action of the steam-sterilized soils. Six temperatures ranging from 17° to 32° C. were again used. This experiment was begun on January 18, 1918, using the White Burley variety in the same soil as previously used, and concluded on February 26. Marked differences in growth on the uninfested and infested soils were already noticeable on February 5; the plants in the sterilized soil 20° to 21° and 23° to 24° were twice as large as those in the infested soil; whereas the plants in the infested and uninfested soil at 31° to 32° were practically equal in size. The final results are shown in Table VI A, in terms of air-dry weight of the stalks, leaves, and roots in the infested and uninfested series, together with the amount of infection on the roots. It may be seen that the best temperature for growth in this case was apparently 28° to 29° for the above-ground portions of the plant, but that the best root development took place in the cooler soil at 23° to 24°. In the infested soil a gradual increase in growth from the lowest to the highest temperature is evident. (Pl. 2, II–III.) Practically the same is true for root development. (Pl. 2, IV.)

EXPERIMENT VIII.—In this experiment an ordinary greenhouse soil mixture, free from *T. basicola* was used. No sterilization, therefore, was used, and infestation with *T. basicola* was produced by thoroughly incorporating a heavy spore suspension of endoconidia of the fungus from young cultures on agar. The experiment was now run as before, except that the Connecticut Havana variety, which is relatively much more resistant to *T. basicola* than the White Burley, was used. The young seedlings were transplanted on March 6, 1918. On March 17 the plants in the inoculated pots already showed signs of heavy infection at the lower temperatures. On days of high transpiration the

plants in the inoculated series wilted first at from 22° to 23° C., but no wilting occurred at 31° to 32° or in the inoculated soil. On April 15 the experiment was terminated, the stalks and roots were cut and dried, and the roots washed out as carefully as possible and dried. The air-dry weights are shown in Table VI B.

TABLE VI.—*Influence of soil temperature on the rootrot of tobacco*

A. WHITE BURLEY VARIETY, NORMALLY INFESTED SOIL

Series.	Temperature of soil.	Average air-dry weight per plant.				Amount of infection in infested soil.
		Stalk and leaves.		Roots.		
		Uninfested soil.	Infested soil.	Uninfested soil.	Infested soil.	
	°C.	Gm.	Gm.	Gm.	Gm.	
1.....	17–18	3.6	0.35	0.32	0.03	Heavy; roots all black.
2.....	20–21	5.4	1.20	.66	.07	Do.
3.....	23–24	7.1	1.70	.86	.10	Not quite as heavy as in series 1 and 2.
4.....	25–26	6.9	2.75	.79	.17	Considerable, but much less than in series 3.
5.....	28–29	7.6	3.8	.70	.16	Slight infection.
6.....	31–32	5.9	5.7	.25	.28	No definite signs of disease.

B. CONNECTICUT HAVANA VARIETY, ARTIFICIALLY INFESTED SOIL

1.....	12–13	1.8	0.53	0.26	0.16	Heavy; very few white roots.
2.....	17–18	7.9	.65	.70	.15	Heavy; about same as in series 1.
3.....	22–23	9.1	1.15	1.05	.19	Heavy but less than in series 1 and 2.
4.....	26–27	10.6	3.75	1.13	.38	Much less than in series 3.
5.....	28–29	10.8	8.10	1.13	.90	Very slight.
6.....	31–32	10.5	7.20	.75	.60	No sign of infection.

The largest yield of the above-ground portions of the plants in the uninoculated series occurred at 28° to 29°, but was only slightly larger than at 26° to 27° or 31° to 32°. The largest root development occurred at 26° to 27° and 28° to 29°, with a decided falling off at 31° to 32°. In the inoculated soil the largest yield of the above-ground parts was at 28° to 29°, with some falling off at 31° to 32°, though not due to infection. It should be noted here that the greater reduction in yield is at 17° to 18°, the disease apparently less marked at 12° to 13° and at 22° to 23°. Practically this same relation holds for the roots. This, together with other experiments, seems to indicate with considerable certainty that the amount of infection and severity of the rootrot are most marked at temperatures ranging between 17° to 23° C. At temperatures below about 15° the extent of the disease is reduced, but this temperature also is too low to permit any growth of tobacco, and consequently is of little practical importance. On the

other hand, at temperatures of 26° and above, the amount of infection and the extent of the injury done are gradually reduced until at about 30° no appreciable injury results, and at at 31° to 32° it is permissible to say that practically no infection whatever occurs.

The results having shown that the rootrot can be practically controlled by high soil temperatures, which at the same time are favorable for the growth of tobacco, the question naturally arises as to how far a plant may recover from serious root infection, provided a change of soil temperature from one favorable to disease to one unfavorable to disease is brought about. Eight White Burley plants which had been planted to the infested tobacco field in June but which had made no appreciable growth during the entire season in the field were taken up late in September with their adhering soil and transplanted into the jars with the infested soil. Four of these were then set in the temperature tanks at a low temperature (20° to 21°) and four at a high temperature (30° to 31°). After remaining at these temperatures for a month the roots were dug out as carefully as possible, and the results are illustrated in Plate 3. The plants had almost no roots when placed in the tank, and one must marvel at the wonderful persistence of tobacco plants in maintaining themselves with an almost complete lack of root system. At the higher temperature, however, new roots were forced out through the blackened bases of the stalks and remained uniformly clean, white, and free from disease. This experiment was repeated with even more striking results by moving jars with badly diseased plants from the low temperatures to the high temperatures in the tanks. In the space of three or four days the plants seemed to have taken on renewed vigor and growth. These experiments seem to prove beyond doubt that similar conditions may happen in the field under practical conditions, and that the phenomena of recovery of a badly diseased crop, so frequently noted in the field within a short period of time, is no doubt due in large measure to natural changes in temperature relations of the soil.

SOIL TEMPERATURES IN THE FIELD

It now remains to ascertain how far the soil temperatures occurring under normal conditions in the field may influence the actual amount of infection and damage from rootrot. It is necessary, therefore, to determine the actual soil temperatures occurring during the growing season in order that a knowledge may be obtained of the change occurring at different times in the same season and during different seasons taken as a whole. Unfortunately not a great many reliable data upon soil temperatures for summer months in various sections of the country exist. Such as do exist, however, may have a bearing upon future studies of the influence of soil temperatures upon the occurrence of disease. It is to be expected that soil temperatures have a fairly con-

stant correlation with air temperatures, and it is highly probable that a fairly constant ratio may be calculated which will enable the extensive data on air temperatures to be used in considering relations of soil temperatures to disease.

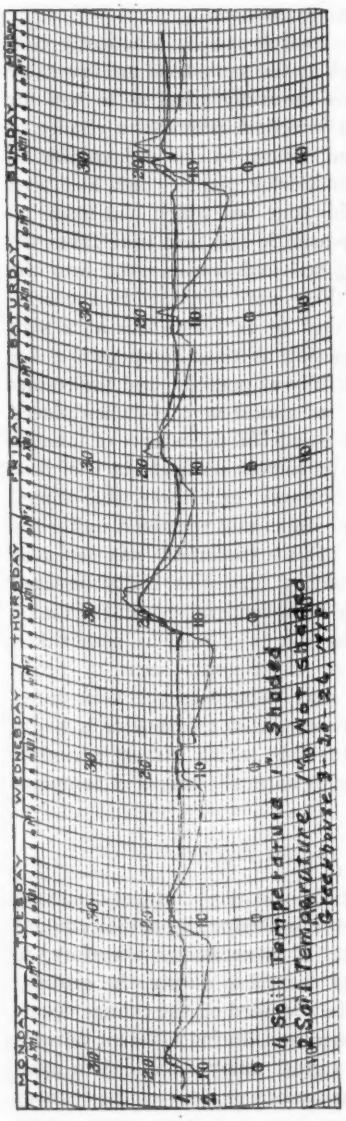

FIG. 1.—Soil-thermograph records showing the influence on soil temperature of the shading of soil by growing tobacco.

The data taken in connection with the studies presented in this paper were started in the spring of 1915. For this purpose electrical resistance thermometers were used. These were buried in the soil in tobacco fields at the Station farm at depths of 2, 4, and 8 inches. Some of the thermometers were buried in such a way that they would record the temperature of soil becoming gradually shaded by the growing tobacco, while with others the soil was exposed continuously to the full sunshine. The latter temperatures are the ones upon which conclusions were drawn, since in a badly infested field shading of soil would be relatively small, owing to the poor growth of the crop. On the other hand, where very heavy infestation does not occur or a relatively resistant variety is used, the relative importance of shading must be considered (fig. 1).

The temperature readings were taken with duplicate thermometers each day at 1 p. m. This hour was selected as it was the most convenient time of the day to take the readings. No great importance, however, can be attached to the time of taking daily readings, on account of the great daily variation which occurs especially near the surface of the soil. It would be most desirable to record the minimum and maximum temperature for each day, but in using electrical thermometers this would

entail too many readings. Temperature readings taken at 7 a. m. and at 1 and 5 p. m. for one week (July 6 to 13, 1916) showed that the temperature was anywhere from 1 to 5 degrees lower at 7 a. m. than at 1 p. m. and to average slightly higher at 5 p. m. than at 1 p. m., indicating that the maximum perhaps was reached at some time between 1 and 5 p. m., and on clear days at about 3 p. m. A recording soil thermograph was also used at a depth of 4 inches in 1917. From these records it may be noted that the highest temperature usually occurred about 4 p. m. (fig. 2).

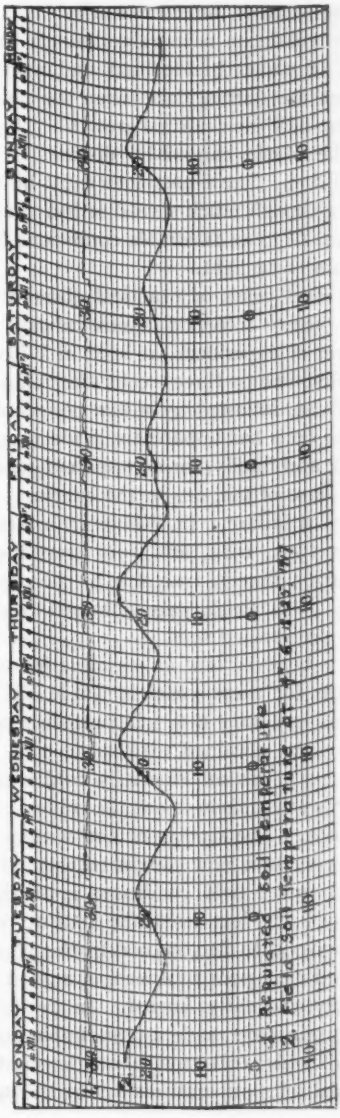

FIG. 2.—Soil-thermograph records giving comparison of a typical record of regulated soil temperature in tanks with a typical record from the field at a depth of 4 inches.

A more important consideration, however, is the general rise or fall of temperature during extended periods of a week or more, or the general trend of the temperature for one season as compared with another.

In the northerly latitudes the growing period of tobacco, practically speaking, lies within the months June, July, and August. Although much tobacco remains in the field during the month of September, practically all the growth must be made before that time. July undoubtedly is the critical month in which most of the growth should be manifested, although if growth is retarded until August, and a warm fall follows, with absence of frost until late into September, a marketable crop may often be produced. In the northern districts nearly all tobacco is planted in June. From the standpoint of temperature this is the most favorable month for the rootrot. Heavy infestation in June followed by a warm July, however,

may overcome the disease. If the warm period is delayed until late July or August, recovery may still be made and a late crop of good yield produced, provided the balance has not swung to the other extreme—that is, forced maturity.

No condition is more commonly seen in infested tobacco fields than that of plants budded out and ready for topping two to three weeks before the normal date when the plants have obtained only one-fourth to one-half their normal growth. This is a direct result of the starvation of the plants caused by disease. A drouth may bring on the same condition. The plants then must be topped when this stage is reached, and although much spread of leaf may subsequently occur, owing to the arrival of more favorable conditions for growth, yet the yield is almost certain to be light.

Several years of practical observation of infested fields have shown that heavy infection almost always occurs in June. Every tobacco grower of experience, at least in Wisconsin, can cite cases where during the first two or three weeks after planting the crop prospects have been excellent, followed by a like period of uncertainty, when the condition of the crop has apparently made no progress or has gone slightly backward, and finally, for no apparent reason, where the crop has taken on a new lease of life, or, on the contrary, has remained to the end more or less of a failure. In Wisconsin a large percentage of poor crops in the years 1913, 1915, and 1917 was due either to poor yield or delayed maturity directly traceable to the rootrot. In the years 1914 and 1916 fairly good yields were obtained, and not much root disease occurred even on infested soils.

It is believed that an examination of the summarized soil temperature records for these years in Table VII, or a glance at the temperature curves in Plates 6–8, will furnish in a large measure an explanation for the results obtained with tobacco in 1915, 1916, 1917, and 1918. The year 1915 was an especially cold season; according to weather bureau records at Madison it was the coldest on record, and also a comparatively wet one. The studies of the writers on the influence of soil moisture, however, have now convinced them that its importance as a controlling factor under field conditions is small as compared with temperature. In 1915 the loss from the rootrot of tobacco was estimated at from $10,000,000 to $20,000,000 in the United States alone. The year 1918 showed very poor prospects of a good crop for a period of several weeks in July and early August. In the latter half of August, however, the Wisconsin crop made a remarkable growth even in the most heavily infested fields; this growth was unquestionably a direct result of the increased soil temperatures during this month.

TABLE VII.—*Average monthly and seasonal soil temperatures for tobacco-growing periods, 1915–1918, at different depths of soil*

Season.	Depth of soil.	Temperatures during month of—			Average for growing period.
		June.	July.	August.	
	Inches.	°C.	°C.	°C.	°C.
1915	2	20.9	20.9	19.0	20.3
	4	20.4	20.6	19.1	20.0
	8	18.0	19.2	18.3	18.5
1916	2	27.6	31.8	23.8	27.7
	4	23.0	27.8	22.7	24.5
	8	18.6	24.3	22.4	21.8
1917	2	21.7	28.2	27.6	25.8
	4	18.7	24.5	24.8	22.7
	8	16.3	21.6	21.5	19.8
1918	2	23.8	27.0	29.0	26.6
	4	21.0	23.6	25.4	23.0
	8	17.9	20.5	21.8	20.1

The practical bearing of this problem is manifold. In so far as seasonal temperatures can be judged and predicted, crop prospects on infested soils can be predicted, and in the northern tobacco-growing sections the infested soils usually constitute anywhere from one-half to three-fourths of the acreage grown. In so far as "warm" soils can be selected—that is, sandy, dark soils with good drainage and a southerly exposure—in preference to "cold" soils, the possible extent of the damage from disease has been reduced. If the crop is planted early on infested soils, heavy infection is more likely to occur in the early stages of plant growth, and the plants will find it more difficult to recover. One of the most common beliefs of the Wisconsin grower, based on observations of several years, is that early planting means plants budding out in July, and an early, light-weight crop. From a purely physiological standpoint there could be only one possible explanation for such behavior of early-set tobacco, namely, the more or less common occurrence of a drouth in July. The inadequacy of such an explanation, however, is shown by the following observations: The vigor of growth of corn and other cultivated crops has remained practically unchecked during many of these frequently recurring so-called drouths in July; likewise, the growth of ordinary tobacco on new soil of a neighboring farm or of a resistant variety in the adjoining row on an infested field has not been greatly interfered with by these weather conditions; finally, exceptionally poor crops of tobacco were grown in Dane County, Wis., in the years 1913 and 1915, whereas the July rainfall was 8.47 inches in 1913 and 5.04 inches in 1915, both greatly in excess of the normal.

It would seem that some value could be attached to late planting on soil infested by *T. basicola*, in view of the low temperatures in June.

On the other hand, the practical application of such a recommendation is doubtful on account of the variations in seasons as to temperature and general growing conditions. In general the farmers must transplant to the fields when the seedlings are of proper size, a matter which usually can not be predetermined very effectively for more than two or three weeks. With steam-sterilized seed beds closer approximations can be made, and seed may be sown two to three weeks later than normally, with fair certainty of obtaining plants by June 20 to 30. Planting later than July 1, however, is no more certain of giving satisfactory final results than early planting.

During the season of 1917 a planting experiment was carried out, with the hope of getting some data on this subject. Seedlings were transplanted at intervals of one week from June 11 to July 23 on infested and uninfested soil. Unfortunately, the White Burley variety was used on heavily infested soil, and the season being relatively cool throughout, no appreciable difference in yield occurred on the infested soil. On the uninfested soil, however, the advantage was all with the early-set tobacco; a gradual decrease in size and value occurred in the later plantings. A wide range of observation has convinced us that, other conditions being alike, early-planted tobacco on uninfested soil usually is considerably safer than late-set tobacco on either infested or uninfested soil.

Before leaving this subject another point of more scientific interest should be considered: Why are tobacco roots most seriously attacked by *T. basicola* at from 17° to 23° C. and practically not at all at a temperature of 30° C.? Several hypotheses may be formulated. The simplest explanation would be that the resistance of the roots to the parasite is modified at different temperatures, high susceptibility occurring from 17° to 23° and practical immunity at 30°. At first sight a tenable theory seemed to be that the increased vigor of root formation at higher temperatures sufficed to overcome the destructive effects of the disease. On the other hand, the action of temperature variation may be regarded as modifying the ability of the fungus to grow in the soil or to attack the host. On the basis of some preliminary experimental results, the latter theory seems to be the most probable.

It should be noted, however, that the behavior of the parasite in culture does not correspond entirely with its behavior on the host as regards temperature relations. Gilbert (*12*) found the following critical temperatures for growth: Minimum 7° to 8° C., optimum 30°, maximum 34° to 37°. The determinations of the writers have given figures very much the same as these. The temperature most favorable for infection does not therefore agree with the optimum for growth in culture. On the other hand, the optimum growth in culture is obtained at 29° to 30°, where the organism is apparently ineffective as a parasite. It is not possible, therefore, to draw any decisive conclusions as to the behavior of the fungus as a parasite from its behavior in artificial culture

media. At temperatures of only 3 or 4 degrees above the optimum in culture, however, the fungus, though making some growth, behaves quite normally, and it is not difficult to conceive of no infection or growth occurring on the host at temperatures above 30°. The results are probably in line with the relation of temperature to infection with other parasites, where it is known that the fundamental factor concerned is that of spore germination.

The following brief description of some experiments may be of interest: The roots of tobacco plants in 7-inch pots were forced to grow out through the perforation in the bottom of the pots by setting them in battery jars partly filled with water. After the roots had made a good start, the jars containing the plants were set in the temperature tanks, at high and low temperatures, 31° to 32° C. and 17° to 18°, respectively. They were allowed to remain there for a week to 10 days. During this time many fresh roots were formed. Two jars at each temperature were now removed to a temperature of 23° to 24°, and young endoconidia of *T. basicola* introduced into the water surrounding the roots. Other plants remaining at 31° to 32° were also inoculated in a similar manner. Good visible infection occurred in 3 to 4 days in all plants at 23° to 24°. No difference was observed at this time or later in the roots which had formed, either at a high or at a low temperature. No infection occurred at 31° to 32° after 8 to 10 days, but infection did occur when the pots were removed to a lower temperature without reinoculation. This showed that the fungus had not been destroyed.

This test at least demonstrated that the increased growth of the host at higher temperatures is not due to the overcoming of the effects of the disease by increased root development, but is due to the inability of the fungus to infect the host. It also tends to show that any resistance or susceptibility at high or low temperatures which the roots have developed is rapidly lost, since infection must have occurred within 24 to 36 hours after changing from the extremes to the medium temperature. There is room for a great deal of investigation, however, upon the intimate environmental relations of host and parasite in this disease, and it is expected that this subject will be treated in more detail in another paper.

INFLUENCE OF ORGANIC MATTER IN THE SOIL

The content of organic matter and humus in the soil has been ascribed by most investigators of tobacco rootrot as being a very influential factor in determining the amount of disease. Practically all the writers agree that the addition to the soil of vegetable matter in the form of green manures or barnyard manures increases the extent of the disease. Massee (*19*) has gone so far as to state that the disease can not occur at all in the total absence of organic matter, since he believes the fungus must gain some stimulus while living as a saprophyte before being able to penetrate the host. Nearly all of these conclusions, however, have

been based on observation rather than on experimental data. The question is an important one from a practical standpoint. Will the selection of soils low in organic matter or avoidance of the use of green or barnyard manures materially aid in reducing the disease?

This is one of the most difficult problems to subject to experimental test in such a way that wholly reliable conclusions can be drawn. It illustrates equally well the fallacy of drawing far-reaching conclusions from mere field observation. It is evident that the organic matter of the soil has profound influence upon a large number of other factors such as water-holding capacity, food supply, temperature, reaction, texture, aeration, and saprophytic growth of microorganisms in the soil. To eliminate all these factors, even in the most carefully controlled experiments, is impossible. To judge of their relative importance in the results obtained in an experiment, however, on the basis of the behavior of such factors from other experimental evidence, is quite likely to yield fairly reliable results.

TABLE VIII.—*Influence of amount of organic matter in soil on rootrot of tobacco*

Series.	Relative percentage of leaf mold to ground quartz by weight.	Experiment I.				Experiment II.			
		Loss on ignition.	Average air-dry weight per plant.		Ratio.	Loss on ignition.	Average air-dry weight per plant.		Ratio.
			Uninfested soil.	Infested soil.			Uninfested soil.	Infested soil.	
		Per cent.	*Gm.*	*Gm.*		*Per cent.*	*Gm.*	*Gm.*	
1	0	0.22	0.88	0.014	1 : 63	0.24	3.35	0.25	1 : 13
2	10	1.65	5.07	.020	1 : 253	1.05	3.35	.42	1 : 8
3	20	.....	.....	.....	.....	2.85	4.90	.60	1 : 8
4	40	5.85	5.93	.052	1 : 114	7.45	5.60	.32	1 : 17
5	60	9.67	7.11	.107	1 : 66	12.7	5.55	.29	1 : 18
6	80	24.1	6.17	.179	1 : 35	23.5	7.00	1.85	1 : 4
7	100	40.8	8.01	.156	1 : 51	52.7	7.27	.70	1 : 10
8	[a] 100	40.8	7.19	.072	1 : 99	.....	.....	.....	.....

[a] Leaf mold heated to 110° C. before infestation.

An attempt was therefore made to arrive at such conclusions by studying the behavior of the disease in the purest ground quartz sand available and also in pure leaf mold, together with mixtures of the two in various proportions (Table VIII). The chief difficulty met with at once in such a combination is to obtain an approximately uniform supply of plant food in these various media. The leaf mold was found to contain sufficient plant food to support normal growth, though after the third crop the plants showed potash hunger. To the pure quartz cultures a nutrient solution sufficient for plant growth was added, and decreasing amounts were added to the various mixtures of sand and leaf mold with a rough estimate of the amount of nutrient salts required. Two pots of each series were then inoculated with *T. basicola* and two

left uninoculated as controls. All were then transplanted with the White Burley variety from sterilized soil. It became evident at once that on uninfested soil the rate of growth of the plants in the extremes of the series was markedly different; the leaf mold was much more favorable for growth than the sand with nutrient solution. Several tests were run on these pots, and also on another series made up in a similar manner (Experiment II, Table VIII). Most of the data concerning them exists as notes and estimates of relative growth during the progress of the experiment. Much reliance can not be placed on the weights, owing to the large variation in fertility, although the ratios given of the growth on infested to that on uninfested soil indicate the general trend of the results.

The experiments, of which there were a considerable number, can not profitably be discussed here in detail. Owing to the variation in results obtained in growth, much reliance was placed on estimates of actual infection on the roots themselves, estimates difficult to express in figures. After summarizing the results of all the tests run (nine in number), it can be stated with considerable confidence that the importance of organic matter in the soil is relatively small, so far as infection and severity of the disease are concerned. It seems, however, that heavy infestation is more rapid, and is more likely to be maintained through unfavorable periods for the parasites in soils high in organic matter rather than in those low in organic matter. Given a uniformly heavy inoculation of the soil with endoconidia of *T. basicola*, the rate and severity of infection is apparently practically the same in pure sand as in the pure leaf mold. Massee's conclusion (*19*) that *T. basicola* is a weak parasite and unable to infect the host except in the presence of organic matter seems entirely unwarranted. This has been further shown by infections obtained from spore suspensions in pure water or spores alone placed directly upon roots grown in a moist atmosphere. After the lapse of a considerable period of time from the date of inoculation, however, it seems certain that *T. basicola* becomes more firmly established in pure leaf mold than it does in pure sand, although this is apparently a difference of amount of infestation and not one of virulence (Pl. 4, V–VI).

With regard to the various mixtures of sand and organic matter, the conclusion seems justified that, so far as infection following inoculation is concerned, it takes place with equal ease in all (Pl. 4, III–IV). The development of infestation of the soil, however, has not given quite the expected results. Doubling or tripling the content of organic matter apparently has not increased infestation, and in some cases increasing the ratio up to 80 parts of leaf mold seemed actually to reduce it. The results, however, have not been sufficiently uniform in this respect to warrant a final conclusion, and it is not certain that factors other than the organic matter do not play a part here. Nevertheless, the fact that increasing the organic content of the soil, two, four, six, and eight times,

on the basis of percentage loss on ignition, has not consistently increased the amount of disease seems to warrant the conclusion that the growers have little to fear in the way of increased infestation of the soil as a result of plowing under green manures or applying 10, 20, or 40 tons of manure to the acre.

INFLUENCE OF THE CLAY AND SAND CONTENT OF THE SOIL

The value of sand or sandy soils in reducing the severity of the rootrot and its increased severity in clay soils has been especially suggested by Benincasa (*2*) and Gilbert (*12*).

A pot experiment with pure quartz sand and with the purest clay obtainable was carried out with the hope of throwing more light upon this subject. The only factor which it is desirable to vary in such an experiment is the size of the soil particles. Although this is not practicable, the relative proportion of sand and clay particles no doubt resembles soil conditions equally well. Superior red clay was obtained from the substation of the State experiment station located at Ashland, Wis. This is a very "heavy" pure clay soil containing very little organic matter and is low in fertility. The sand used was a medium to coarse ground quartz. The mixtures of sand and clay made were those shown in Table IX.

TABLE IX.—*Influence of relative amount of clay and sand on rootrot of tobacco*

Series.	Mixture.		Average air-dry weight (in grams) of plants.			
	Sand.	Clay.	Experiment IV.		Experiment V.	
			Uninfested soil	Infested soil.	Uninfested soil.	Infested soil.
1.....	All.....	None.....	1.60	0.40	0.75	0.26
2.....	Three-fourths....	One-fourth.....	1.0	.25	.61	.20
3.....	One-half.....	One-half.....	.90	.20	.52	.16
4.....	One-fourth.....	Three-fourths....	.75	.25	.50	.25
5.....	None.....	All.....	1.10	.15	.30	.13

The experiment in this case was confronted with practically the same difficulties and complications as occurred in the tests with organic matter. The clay soil alone, or in mixtures with sand, seemed to have an "injurious" action upon the growth of tobacco which was not remediable with nutrient solutions applied. The yield, therefore, was low in all cases. Two pots of each series were inoculated with equal volumes of spore suspension from cultures of *T. basicola* on agar, which were thoroughly mixed with the soils. White Burley tobacco was then transplanted into them. The first experiment was started November 18, 1916. The result of this experiment was not recorded by weight, but some of the crocks were photographed (Pl. 5, I), and serve to illustrate the results obtained. The conclusion drawn from this experiment was that

sand was considerably more favorable to infection of roots with *T. basicola* than was clay.

Another test with the Maryland broadleaf variety, started on October 13, 1917, gave practically the same results; root examination showed the greatest infection with sand, less with an admixture of one-fourth clay, and almost none with one-half clay, and still less with larger amounts of clay. However, in one pot heavy infection occurred at the base of one plant, and the results were interfered with somewhat by nematode injury.

All the soils were then resterilized with steam and two pots of each series heavily infested with a suspension of young endoconidia of *T. basicola* from agar slants. White Burley was again replanted into all the crocks. The results obtained in this case differed somewhat from the preceding, owing most probably to heavier infestation. Infection seemed to occur most rapidly and severely in the one-half and three-fourths clay mixtures, but in a few days the plants in the infested series were practically identical in appearance, and after about four weeks, nearly all were killed.

These were now removed, and a more resistant variety, Connecticut Havana, transplanted into the pots. After 18 days all these also were practically equally diseased in the infested series, and were not quite half the size of those in the uninfested series. After about six weeks' growth these plants were cut and the air-dry weights determined as shown in Table IX, Experiment IV. The pots were again planted to tobacco with similar results (Table IX, Experiment V). The results of the last experiments seem to indicate that in the presence of heavy infestation of the soil very little difference exists between clay and sand mixtures in the severity of infection of tobacco by *T. basicola*.

The results obtained in the first experiments are believed to be due to the fact that the parasite found clay soils unfavorable for growth and multiplication, and especially for penetration of mycelium as compared with the sand, and therefore less actual infection occurred. On the other hand, with heavy infestation sufficient spores were in close proximity to the roots to produce good infection at once.

With respect to the persistence of *T. basicola* in soil, and its gradually increasing infestation in spite of unfavorable conditions, it is believed that clay soils may eventually be more injurious than sandy soils, but the results seem to justify the conclusion that from the standpoint of texture alone, the selection of loose sandy soils, or the use of clay soils does not necessarily predetermine to any important degree what the injury from *T. basicola* will be. It should be added that clay soils draining more poorly and warming up more slowly, undoubtedly may be considerably more harmful than sandy soils, because of the influence of saturated soils and low temperature upon the severity of the rootrot. It is also believed that the tendency of the clay soils toward greater compactness may also favor somewhat the occurrence of the disease.

INFLUENCE OF SOIL FERTILITY

In the case of soil-infesting parasites which cause the loss of large portions of the root system in such a way that it can no longer function normally for the benefit of the plant, it seemed probable that the quantity of available plant food would influence growth in infested soil. It may be supposed, for instance, that, if in a soil low in fertility one-half of the roots are destroyed by disease, doubling the quantity of available plant food would materially reduce the actual amount of damage done in yield of crop. This is in accord with the conclusions of Briggs (*3*) on this subject. On the other hand, there are the views expressed by many pathologists with respect to various diseases, and also by Gilbert (*12*) and others for the Thielavia rootrot, that an increase of fertilizers, especially those of nitrogenous nature, renders the plant more susceptible to attack. Aside from these theoretical conclusions, we are confronted with the facts that the tobacco rootrot as it occurs in the field is not confined especially to soils in a low or high state of fertility, and that the application of fertilizers, whether as barn manure or commercial fertilizer, seems to have no marked effect upon the relative amount of disease, or on the growth of the plants in infested soil in seasons favorable to rootrot. These conclusions are based on four years of fertilizer plot experiments carried on at Edgerton, Wis., during the years 1910 to 1913, inclusive, the detailed results of which can not be given here. These experiments were planned to determine if it is possible to remedy the "worn-out" or "deteriorated" condition of old tobacco soils by the use of fertilizers of various sorts, although the full significance of *T. basicola* as the cause of this condition was not recognized at the time the experiments were started, and it was not until after three years of failure to obtain any marked results with fertilizer treatments on a wide variety of old soils, coupled with highly beneficial results on soil new to tobacco that the importance of *T. basicola* in crop production was fully realized.

An experiment to determine more carefully the effect of plant food applied in the form of pure salts on the severity of the rootrot was carried out in pot tests in the greenhouse during the winter of 1917–18. Twenty-four 2-gallon crocks with a drainage perforation at the base were each filled with 10 kgm. of soil infested with *T. basicola* from the old tobacco field on the station farm. Twelve of these were now sterilized by steam to destroy the fungus. The cultures were divided into six series of four pots each, two containing infested and two uninfested soil. A complete fertilizer was made up from chemically pure salts according to a formula used for nutrient water cultures, as follows:

	Gm.
Calcium nitrate	40
Potassium chlorid	10
Magnesium sulphate	10
Tribasic potassium sulphate	10

This fertilizer was added to each pot in each series and thoroughly mixed with the soil in the amounts shown in Table X; the application ranged from one which was considered only light, to one which was so heavy as to decrease materially the yield. In these pots three successive crops were grown; the first being the susceptible White Burley; the second the semi-resistant Connecticut Havana; and the third the susceptible Maryland Broadleaf variety. The average air-dry weights for the plants in the infested and the uninfested soil for the three crops are given in Table X:

TABLE X.—*Influence of fertilizer on rootrot of tobacco*

Series.	Fertilizer added.	Average air-dry weight (gm.) per plant.					
		First crop (White Burley).		Second crop (Connecticut Havana).		Third crop (Maryland Broadleaf).	
		Uninfested soil.	Infested soil.	Uninfested soil.	Infested soil.	Uninfested soil.	Infested soil.
	Gm.						
1		7.65	0.95	7.97	4.62	5.60	0.52
2	3.5	7.63	2.25	12.05	3.87	5.25	.60
3	7.0	8.90	.57	11.75	3.60	5.85	.45
4	14.0	11.57	.17	12.17	3.75	9.40	.40
5	28.0	8.15	.48	11.00	1.50	16.80	.31
6	56.0	3.80	.18	6.10	.59	6.42	.15

It may be observed at once that the uninfested soil responded to the fertilizer treatment; the maximum yield for the first and second crops was in the pots which received 14 gm. of fertilizer. Doubling the amount of salt, however, decreased the yield, and quadrupling it acted very injuriously, presumably owing to increased concentration of the soil solution. Very poor growth was made on the infested soil in all cases (Pl. 5, II–III). In the first experiment, though, the lowest application of fertilizer apparently increased the growth, followed, however, by a decrease at higher applications. The most striking fact was that the most beneficial application on the uninfested soil showed no signs of such beneficial action on the infested soil.

The results obtained with a more resistant variety as a second crop are believed to be more representative. In this case there is a gradual decrease in yield with the application of the nutrient salts on infested soil; no increase from the application of 3.5 gm. of fertilizer occurred as in the first crop grown. This influence of a light application of fertilizer to infested soils is apparently in need of further investigation on a wide range of soils and with different varieties. From a practical standpoint it seems safe to conclude that fertilizer as such is wasted when applied for tobacco on soils badly infested with *T. basicola*, and that it may, in fact, do more harm than good.

Theoretically we are concerned with the reasons for the injurious action of nutrient salts on the growth of tobacco in infested soil, or more directly, the increased severity of the disease in the presence of increased supply of nutrient salts. A root system reduced by *T. basicola* evidently is not able to increase its functions in the presence of increased fertility, in substitution for the lost roots; but it still seems as though this should be possible, provided other factors do not interfere.

There are at least three plausible explanations for the observed behavior: (1) the increased concentration of the soil solution may favor fungus growth; (2) increased food supply may favor increased susceptibility to disease; (3) the reduced root system in the presence of increased concentration of soil solution may not have been able to furnish a sufficient supply of water to the plants. The first explanation seems most plausible and yet seemingly can not wholly account for the results obtained. The second, that of increased susceptibility, seems least plausible, since no one has yet satisfactorily shown that actual susceptibility to disease is increased by increased fertilization. There is some reason to suppose that the third hypothesis is a factor. In the uninfested soil a good illustration of the injurious action of high concentration of soil solution on plant growth is found. This is explained as an osmotic relation, the entrance of water to the plant being reduced, owing to the high concentration of the soil solution. It seems probable, therefore, that in the presence of a greatly reduced root system this condition would be exaggerated with a resultant reduced growth. The water-relation theory is strengthened by the observations on the relative time and extent of wilting of plants on days favoring high transpiration. Wilting of tobacco plants during periods of high transpiration on infested soils and rapid recovery is quite common and indicates a delicate water relation existing between the plant and the soil.

INFLUENCE OF COMPACTNESS OF SOIL

Field observations have seemed to indicate that in many instances where the soil in infested fields is hard or compact, owing to poor preparation of the soil or to other causes, the damage from *T. basicola* is more marked than in loose soils. In fact, many farmers have been found who have attributed poor yields to compact soils alone, when, as a matter of fact, rootrot was undoubtedly the primary cause. It is, however, very difficult to say just how much injury is due directly to the hard compact or baked condition of the soil and how much is due to its influence on the progress of the rootrot when present.

A simple experiment to determine this point was carried out. The naturally infested soil from the field was carefully screened and mixed in a relatively moist condition. A 6-inch clay pot was filled with the soil in as loose a condition as possible. This held 2,000 gm. Another pot was then filled with the same soil, with as heavy tamping as possible.

It contained 3,200 gm. Other pots were now filled with 2,900, 2,600, and 2,300 gm. of soil, four pots of each degree of compactness being used. Two pots of each series were steam-sterilized. Connecticut Havana tobacco was then transplanted into them. To avoid any abnormal conditions due to transplanting, especially in the compacted soils, which were so hard that a knife could scarcely be inserted into them, a small amount of soil was taken out of the center of each pot with a cork borer. The hole was filled with uninfested loose soil, and the young plants were transplanted into it so as to give all an equal chance to start.

The results were very interesting. The plants in the sterile compacted soils did very much better than was expected, although the loose soils were much more favorable to growth. In the infested soil, however, the plants in the compacted soil made no growth whatever (Pl. 5, IV). Examination of the roots showed that in the loose infested soil comparatively few lesions occurred, and the taproot was present, while with increasing compactness the taproot was lost, and gradually increasing numbers of lesions occurred.

The experiment has an important practical bearing on the preparation and cultivation of tobacco soils infested with *T. basicola*. Anything which can be done to avoid or remedy compactness or baking of soils will no doubt lessen the disease even in badly infested soils. In other words, soil in good tilth is less likely to be heavily damaged by *T. basicola* than soil in poor tilth.

TRANSPLANTING DISEASED SEEDLINGS

The influence of the use of diseased seedlings for transplanting to the field is not strictly an environmental feature of the problem. It relates, however, to the amount of infestation in the soil and has a very important practical bearing on results obtained under field conditions. It is, furthermore, a point upon which some contradictory evidence has been obtained by various experimenters, especially Benincasa (*1*), Gilbert (*12*), and Clinton (*8*). The results already presented, especially in regard to the influence of soil temperature, may serve to explain the variation in results from year to year, or of the recovery of infected transplanted seedlings. This is, however, apparently not the only explanation. The writer has shown that varieties of tobacco and even strains, vary in their resistance to rootrot (*14*). Transplanting healthy plants to infested soil under favorable environmental conditions for disease is shown to result in marked differences in yield of the different types used.

It is known that the tendency of diseased plants is to send out new roots to replace those lost by disease. Transplanting diseased plants consists practically in infesting a small area of soil surrounding the base of the plant with *T. basicola*. Part of the new roots, especially those

in the early stages of growth, must penetrate this infested soil before reaching large areas of uninfested soil. The ability to resist the disease will therefore determine roughly the number of roots and rapidity with which they pass through this infested area and become established, and should be roughly proportional to the resistance of the different varieties under similar environmental conditions.

To determine the influence of transplanting varieties differing widely in relative resistance to the rootrot, 11 such varieties were sown in a seed bed infested with *T. basicola*, and also in a sterilized bed as controls. The relative resistance in the seed bed is about the same as that in the field. The susceptible varieties especially did very poorly in the infested beds, but most of them reached a sufficient size for transplanting. About 40 plants of each variety from infested soil and the same number from uninfested soil, were then transplanted side by side in uninfested soil (Pl. 5, V). The results are given in Table XI in which the green weights of 25 healthy plants and 25 diseased plants of each variety are shown, together with the decreases in weight due to the use of infested seedlings. It will be noted that a reduced yield occurred in all cases, but whereas the disease was small in the case of varieties known to be resistant to *T. basicola* it was relatively very high in those varieties which are susceptible. The results are not exactly comparable on this basis on account of the difference in yield of varieties under normal conditions but serve to illustrate the point in question.

TABLE XI.—*Influence of transplanting diseased tobacco plants in uninfested soil*

Variety.	Weight of 25 green plants.		
	Healthy plants.	Infected plants.	Decrease due to disease.
	Gm.	*Gm.*	*Gm.*
White Burley	66. 50	25. 00	41. 50
Maryland Broadleaf	65. 00	38. 00	27. 00
Big Oronoco	57. 75	32. 50	25. 25
Yellow Pryor	59. 00	35. 50	23. 50
Pennsylvania Broadleaf	82. 50	27. 25	55. 25
Kentucky Greenleaf	49. 75	33. 50	16. 25
Italia Kentucky	60. 00	49. 00	11. 00
"Pease Seed"	49. 00	40. 00	9. 00
Ohio Seedleaf	70. 50	51. 00	19. 50
"Northern Hybrid"	65. 00	55. 00	10. 00
Brasile Beneventano	56. 50	53. 00	3. 50

Gilbert (*12*) reports an experiment in which "Havana Broadleaf" tobacco was used and in which the yield from infected and healthy plants was practically identical. By "Havana" Broadleaf was meant, it is presumed, the relatively resistant variety better known as "Connecticut Broadleaf." If environmental conditions were favorable for the

occurrence of disease, different results no doubt would have occurred had Gilbert used a more susceptible variety.

Practical advice on the use of infected seedlings will, then, vary with the variety used. Infected seedlings should never be used if it is possible to avoid it, especially on soils which are not infested, since this will only hasten the time when all the soil will become so thoroughly infested as to make a change to newer soils necessary. On the other hand, it frequently is necessary to risk infected plants, as others may be unobtainable. In such instances it is much less likely that serious injury will result if the infection is on a resistant variety. Again, it should be remembered that infected seedlings of even a susceptible variety transplanted into a heavily infested soil may produce a normal crop under favorable conditions, such as a very warm season and a relatively high soil temperature persisting for a long time.

SUMMARY

(1) The rootrot of tobacco, caused by *Thielavia basicola*, is marked by the stunting of plants in various degrees due to a reduced root system. The extent of the damage is determined in a large measure by the environmental conditions surrounding the roots of the host.

(2) A study of these environmental conditions is essential to the proper understanding of the occurrence and distribution of the disease in general and local areas, and to good judgment in recommendation for control measures.

(3) There seems to be no variation in the pathogenicity of the rootrot fungus secured from different sources. The amount of disease is determined entirely by the susceptibility of the host, the amount of infection, and the soil environmental factors surrounding the roots of the host.

(4) The factors especially studied were the amount of infestation in the soil, the soil moisture, soil temperature, soil reaction, physical structure, and fertility. An analysis of these factors separately as related to rootrot frequently is very difficult, if not impossible. Under normal conditions the end result in injury by rootrot is the sum total of the favorable and unfavorable action of these factors on the disease. Some of these factors are much more important than others.

(5) Other factors aside, the extent of infection and injury from tobacco rootrot is directly proportional to the amount of infestation of the soil.

(6) Rootrot is seemingly capable of developing in relatively dry soils. Increasing the moisture content of the soil up to three-fourths of its water-holding capacity does not materially increase rootrot. Saturated soils are, however, considerably more favorable for the disease than unsaturated ones.

(7) The temperature of the soil is undoubtedly the most important factor determining the extent of the rootrot of tobacco, other factors

being equal. The most favorable temperature for the disease ranges from 17° to 23° C. Below 15° the disease is less marked, and above 26° the severity is gradually reduced, until at about 29° or 30° it has little or no influence. At 32° practically no infection occurs even in the most heavily infested soils. Soil temperature records in the field for four seasons indicate that occurrence of the disease under practical conditions is determined primarily by soil temperature.

(8) The disease is checked by very high soil acidity. Heavy infection can occur, however, in soils showing a considerable acid reaction. The results depend a great deal upon the susceptibility of the variety used in the test, the amount of infection, the soil temperature, and on other factors. The results of tests of Wisconsin tobacco soils indicate that the use of acid fertilizers will not reduce infection by *T. basicola*. Although alkaline soils are more favorable to disease than very acid ones, the use of lime on infested soils may not necessarily reduce the yield due to increased infection from *T. basicola*.

(9) The amount of organic matter present or introduced into the soil does not play a very important part in the amount of infection. High organic matter content, however, no doubt favors increased infestation and aids the fungus to persist in the soil. Where heavy inoculation is made, infection apparently occurs more readily in pure sand than in the presence of organic matter, but under conditions unfavorable for the parasite the amount of infestation is more rapidly reduced in soils lacking in organic matter.

(10) Clay soils as such seemingly are no more favorable for infection than sand, and under certain conditions possibly less so. Clay may, however, favor the persistence of the parasite in the soil, and may actually favor infection because of increased danger of saturation with water and because of the occurrence of lower temperatures than in sandy soils.

(11) Increasing the fertility of infested soil by pure chemicals is likely to cause increased stunting of growth rather than increased growth, especially if too high a concentration of soil solution results. Fertilizers applied to heavily infested soils under practical conditions seem to be largely wasted except in seasons in which such high temperatures result that the disease is held in check.

(12) Field observations and limited laboratory experiments seem to show that infested soils when compacted are more favorable for the disease than loose, open soil.

(13) Transplanting infected seedlings to an uninfested field is a bad practice, although recovery from the disease may occur. Such recovery, environmental conditions aside, is proportional to the resistance of the type used.

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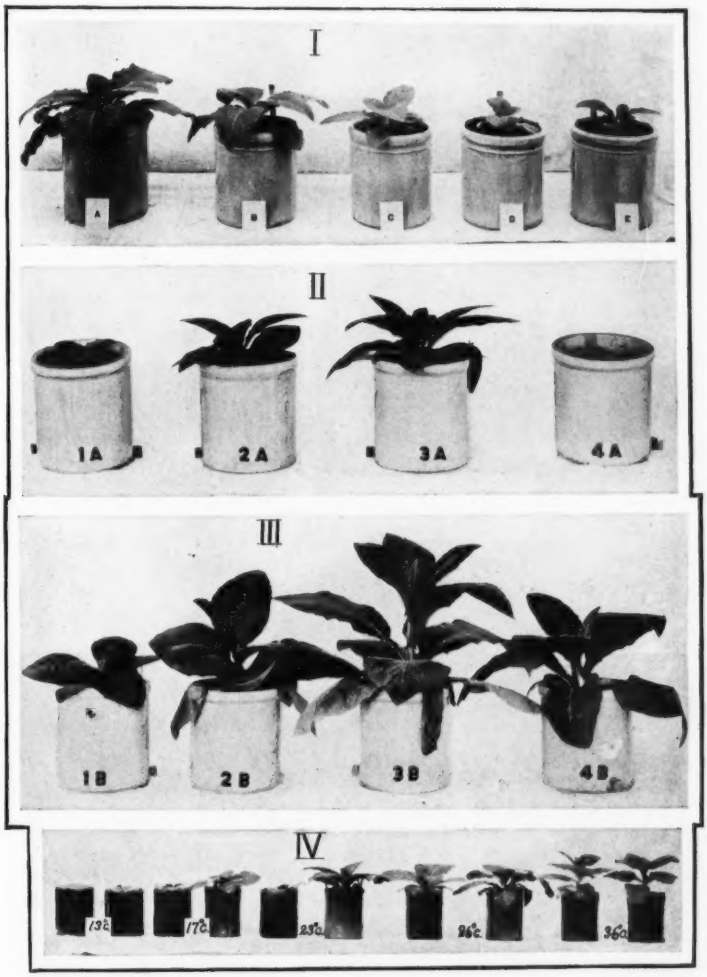
PLATE 1

I.—Influence of amount of infestation on injury from tobacco rootrot: A, All uninfested soil; B, three-fourths uninfested soil; C, one-half uninfested soil; D, one-fourth uninfested soil; E, all infested soil.

II, III.—Influence of moisture content of soil on the amount of injury done by the tobacco rootrot; II, infested soil; III, uninfested soil (control series)—

1A, one-fourth saturation infested soil;
2A, one-half saturation infested soil;
3A, three-fourths saturation infested soil;
4A, full saturation infested soil;
1B, one-fourth saturation uninfested soil;
2B, one-half saturation uninfested soil;
3B, three-fourths saturation uninfested soil;
4B, full saturation uninfested soil.

IV.—Influence of soil temperature on the growth of tobacco in infested soil (jars to left of temperature labels) and in uninfested soil (jars to right of temperature labels) at temperatures of approximately 13°, 17°, 23°, 26°, and 36° C.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D
E
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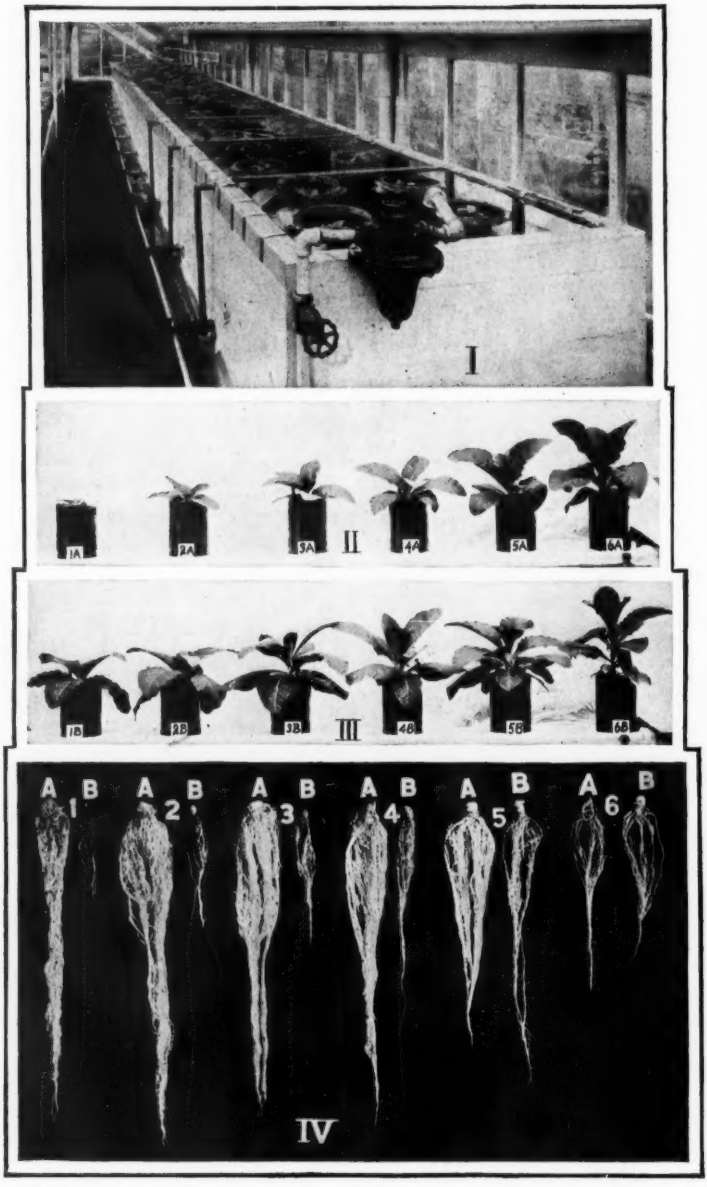
2A
3A
4A
III
1B
2B
3B
4B
IV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2A
3A
II
4A
5A
6A
1B
2B
3B
III
4B
5B
6B
A B A B A B A B A B A B
1 2 3 4 5 6
IV

PLATE 2

I.—Soil temperature tanks used in the temperature experiments. The water surface was later covered with sheet metal and asbestos board.

II, III.—Influence of soil temperature on the growth of tobacco:

1A, infested soil, 17°–18° C.;
2A, infested soil, 20°–21° C.;
3A, infested soil, 23°–24° C.;
4A, infested soil, 25°–26° C.;
5A, infested soil, 28°–29° C.;
6A, infested soil, 31°–32° C.;
1B, uninfested soil, 17°–18° C.;
2B, uninfested soil, 20°–21° C.;
3B, uninfested soil, 23°–24° C.;
4B, uninfested soil, 25°–26° C.;
5B, uninfested soil, 28°–29° C.;
6B, uninfested soil, 31°–32° C.

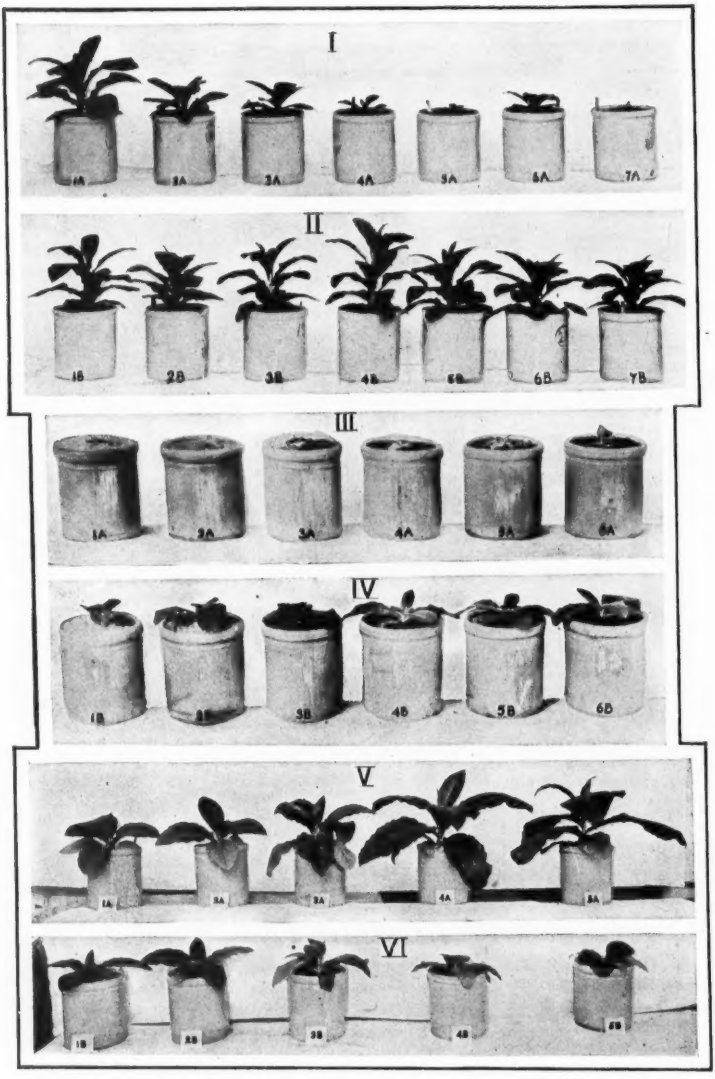
IV.—Influence of different soil temperatures on root development:

1A, uninfested soil, 17°–18° C.;
1B, infested soil, 17°–18° C.;
2A, uninfested soil, 20°–21° C.;
2B, infested soil, 20°–21° C.;
3A, uninfested soil, 23°–24° C.;
3B, infested soil, 23°–24° C.;
4A, uninfested soil, 25°–26° C.;
4B, infested soil, 25°–26° C.;
5A, uninfested soil, 28°–29° C.;
5B, infested soil, 28°–29° C.;
6A, uninfested soil, 31°–32° C.;
6B, infested soil, 31°–32° C.

PLATE 3

Influence of high (30° C.) and low (20° C.) soil-temperature on recovery of plants in infested soil. Both plants were taken from the field where they had made very little growth during the season and placed in temperature control tanks, the roots at 30° being like those at 20° at the beginning of the experiment.

30° C.
20° C.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2A
3A
4A
5A
6A
7A
II
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2B
3B
4B
5B
6B
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III
1A
2A
3A
4A
5A
6A
IV
1B
2B
3B
4B
5B
6B
V
1A
2A
3A
4A
5A
VI
1B
2B
3B
4B
5B

PLATE 4

I, II.—Influence of soil reaction on extent of damage by tobacco rootrot: I, Infested soil; II, uninfested soil—

1A, infested soil, lime requirement 9.38 tons per acre;
2A, infested soil, lime requirement 7.19 tons per acre;
3A, infested soil, lime requirement 4.60 tons per acre;
4A, infested soil, lime requirement 2.62 tons per acre;
5A, infested soil, lime requirement 0.72 ton per acre;
6A, infested soil, slightly alkaline;
7A, infested soil, strongly alkaline;
1B, uninfested soil, lime requirement 9.38 tons per acre;
2B, uninfested soil, lime requirement 7.19 tons per acre;
3B, uninfested soil, lime requirement 4.60 tons per acre;
4B, uninfested soil, lime requirement 2.62 tons per acre;
5B, uninfested soil, lime requirement 0.72 ton per acre;
6B, uninfested soil, slightly alkaline;
7B, uninfested soil, strongly alkaline.

III, IV.—Influence of the amount of organic matter in the soil on injury by tobacco rootrot: III, 1A–6A, Influence of gradually increasing amounts of organic matter in infested soil from 1A, no organic matter, to 6A, all leaf mold.

Planted soon after heavy inoculation.

IV.—1B–6B, Influence of gradually increasing amounts of organic matter in uninfested soil from 1B, no organic matter, to 6B, all leaf mold (control series).

Planted soon after heavy inoculation.

V, VI.—Influence of the amount of organic matter in the soil on injury by tobacco rootrot: V, 1A–5A, Influence of gradually increasing amounts of organic matter in uninfested soil from 1A, no organic matter, to 5A, all leaf mold (control series); VI, 1B–5B, Influence of gradually increasing amounts of organic matter in infested soil from 1B, no organic matter, to 5B, all leaf mold.

Planted some months after moderate inoculation.

PLATE 5

I.—Influence of relative amount of sand and clay on tobacco rootrot: A, uninfested series: B, infested series—

1 A, uninfested soil, three-fourths clay and one-fourth sand;
1 B, infested soil, three-fourths clay and one-fourth sand;
2 A, uninfested soil, one-half clay and one-half sand;
2B, infested soil, one-half clay and one-half sand;
3A, uninfested soil, three-fourths sand;
3B, infested soil, three-fourths sand;
4A, uninfested soil, all sand;
4B, infested soil, all sand.

II, III.—Influence of soil fertility on amount of tobacco rootrot: II, infested series; III, uninfested series—

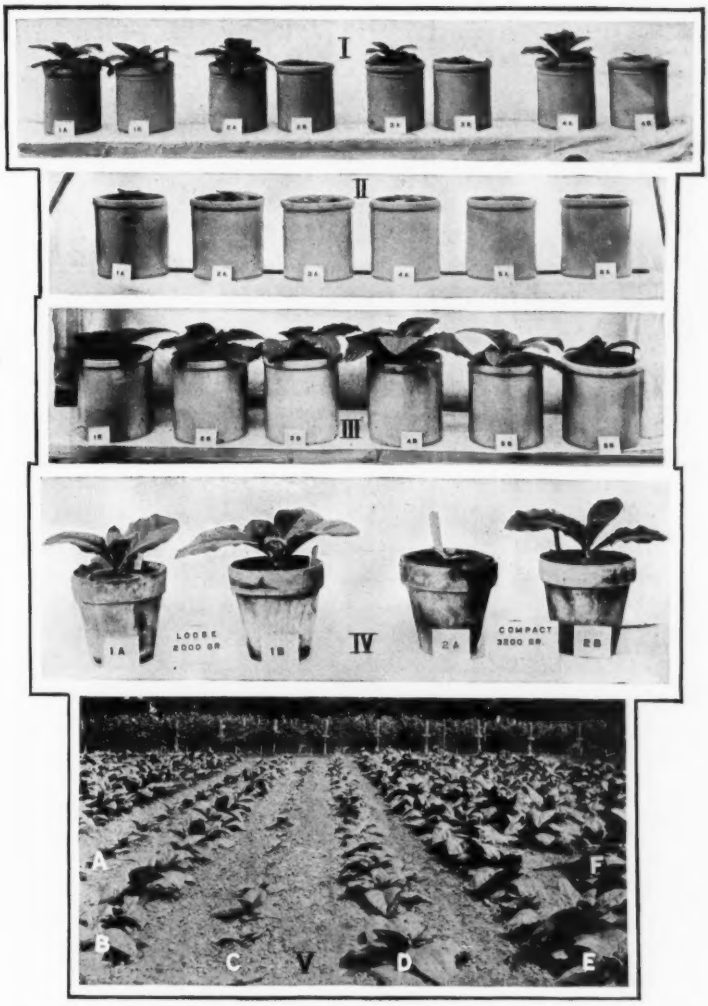
1A, infested soil, no treatment;
2A, infested soil, 3.5 gms. of nutrient salts;
3A, infested soil, 7.0 gms. of nutrient salts;
4A, infested soil, 14.00 gms. of nutrient salts;
5A, infested soil, 28 gms. of nutrient salts;
6A, infested soil, 56 gms. of nutrient salts.
Note increasing injury from nutrient salts beginning at pot 3A.
1B, uninfested soil, no treatment;
2B, uninfested soil, 3.5 gms. of nutrient salts;
3B, uninfested soil, 7.0 gms. of nutrient salts;
4B, uninfested soil, 14.00 gms. of nutrient salts;
5B, uninfested soil, 28 gms. of nutrient salts;
6B, uninfested soil, 56 gms. of nutrient salts.
Note injury from nutrient salts in pots 5B and 6B.

IV.—Relation of compactness of soil to injury caused by *Thielavia basicola:*

1A, infested soil, loosely packed;
1B, uninfested soil, loosely packed;
2A, infested soil, very compact;
2B, uninfested soil, very compact.

V.—Influence of transplanting infected seedlings in healthy soil:

A, Pennsylvania Broadleaf infected seedlings;
B, Pennsylvania Broadleaf healthy seedlings;
C, White Burley infected seedlings;
D, White Burley healthy seedlings;
E, Northern Hybrid (a resistant type) infected seedlings;
F, Northern Hybrid (a resistant type) healthy seedlings.


I
II
III
IV
1A
LOOSE
2000 GR.
1B
2A
COMPACT
3200 GR.
2B
A
B
C
V
D
E
F

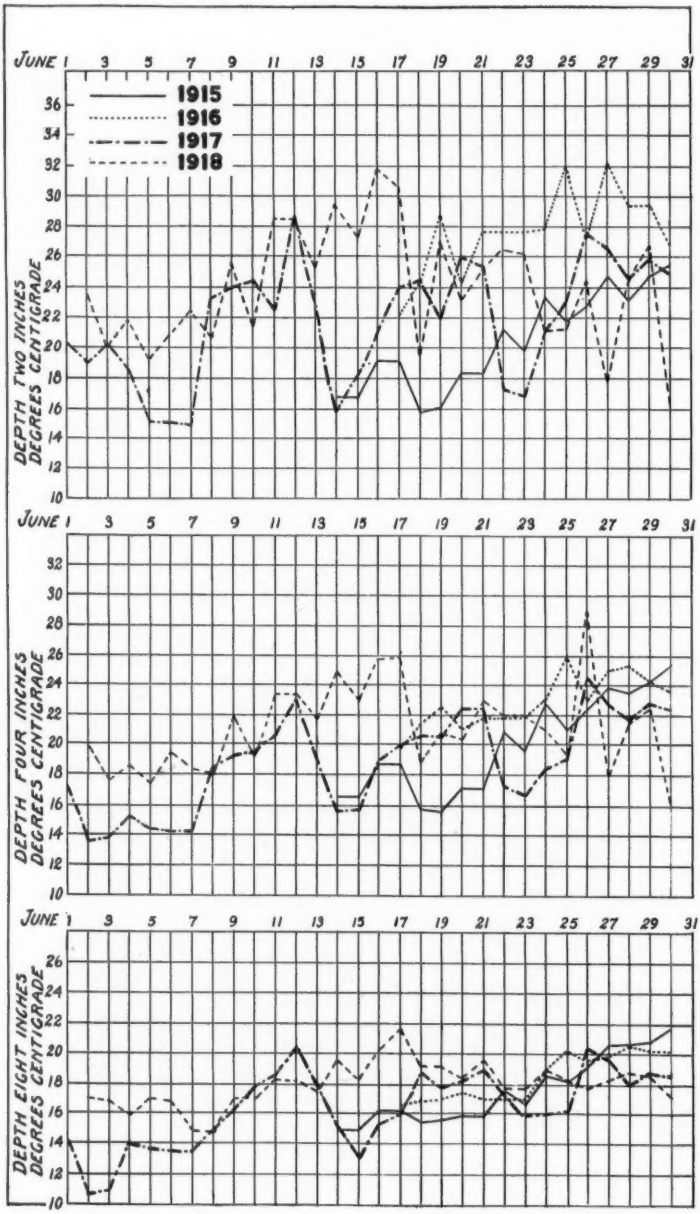
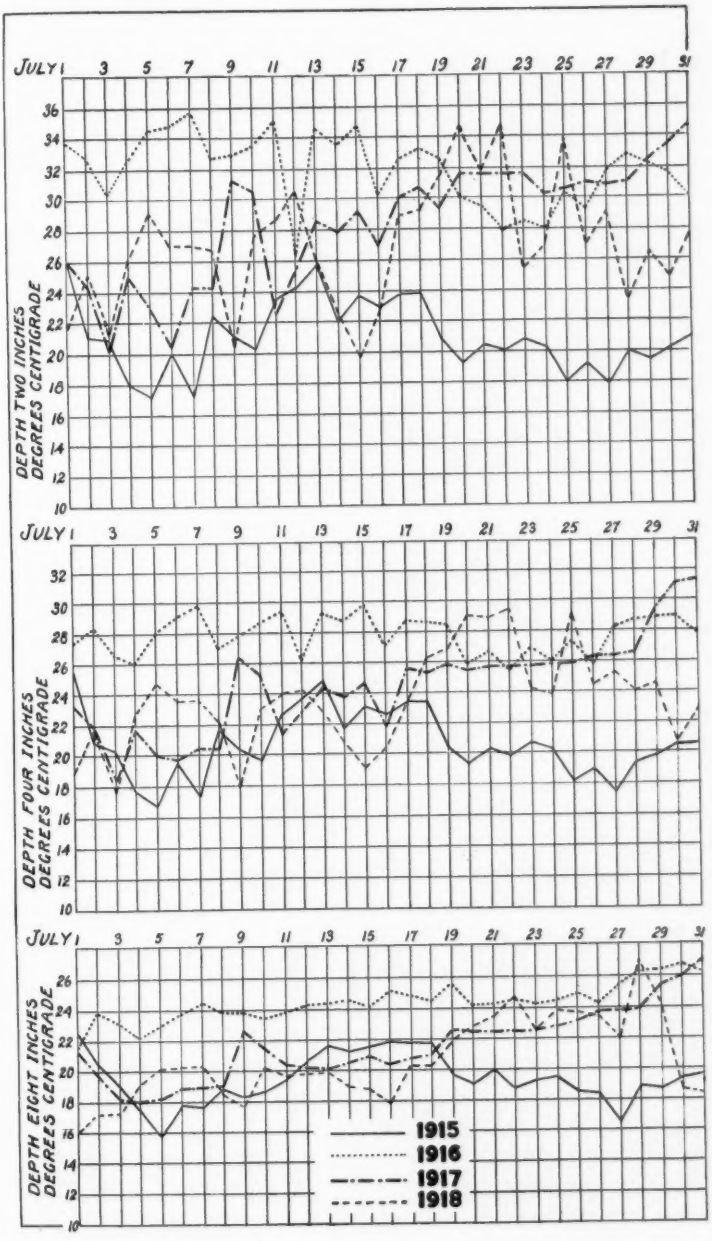

JUNE 1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
1915
1916
1917
1918
DEPTH TWO INCHES
DEGREES CENTIGRADE
36 34 32 30 28 26 24 22 20 18 16 14 12 10
JUNE 1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
DEPTH FOUR INCHES
DEGREES CENTIGRADE
32 30 28 26 24 22 20 18 16 14 12 10
JUNE 1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
DEPTH EIGHT INCHES
DEGREES CENTIGRADE
26 24 22 20 18 16 14 12 10

PLATE 6

Soil temperature graphs for the month of June, 1915–1918, inclusive, at depths of 2, 4, and 8 inches.

PLATE 7

Soil temperature graphs for the month of July, 1915–1918, inclusive, at depths of 2, 4, and 8 inches.


JULY 1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
DEPTH TWO INCHES DEGREES CENTIGRADE
DEPTH FOUR INCHES DEGREES CENTIGRADE
DEPTH EIGHT INCHES DEGREES CENTIGRADE
1915
1916
1917
1918

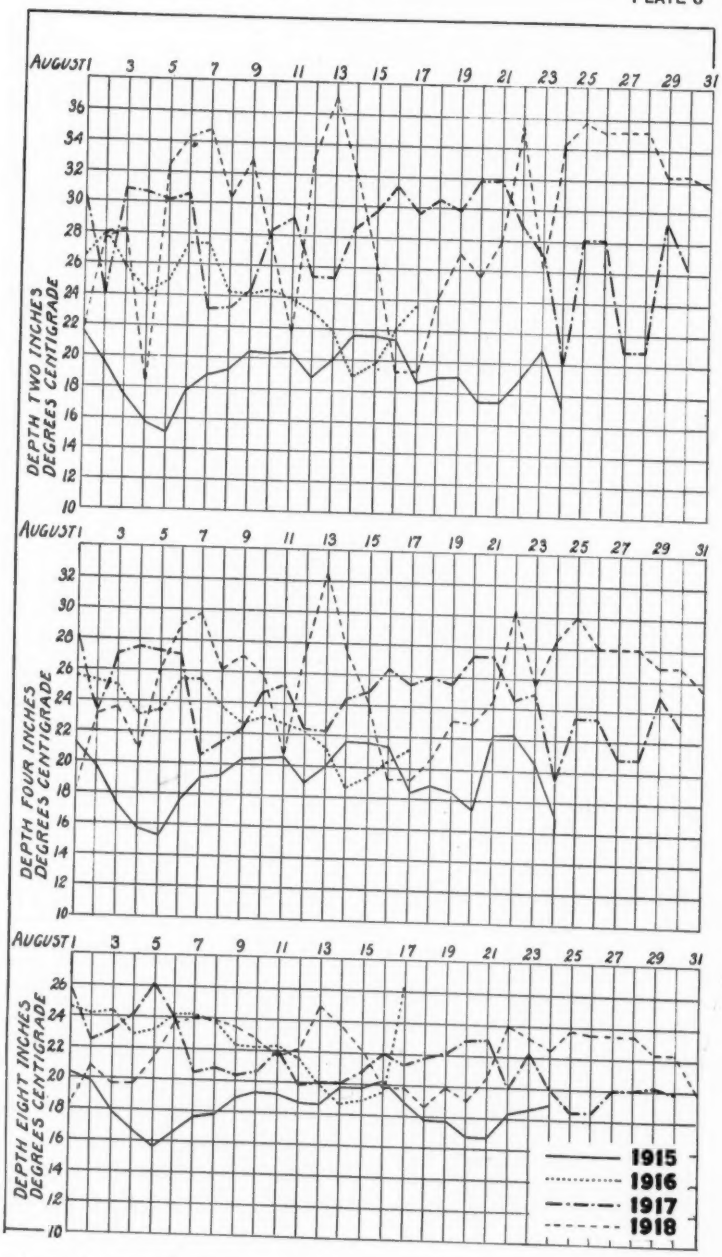

AUGUST 1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
DEPTH TWO INCHES DEGREES CENTIGRADE
36 34 32 30 28 26 24 22 20 18 16 14 12 10
AUGUST 1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
DEPTH FOUR INCHES DEGREES CENTIGRADE
32 30 28 26 24 22 20 18 16 14 12 10
AUGUST 1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
DEPTH EIGHT INCHES DEGREES CENTIGRADE
26 24 22 20 18 16 14 12 10
1915
1916
1917
1918

PLATE 8

Soil temperature graphs for the month of August, 1915–1918, inclusive, at depths of 2, 4, and 8 inches.